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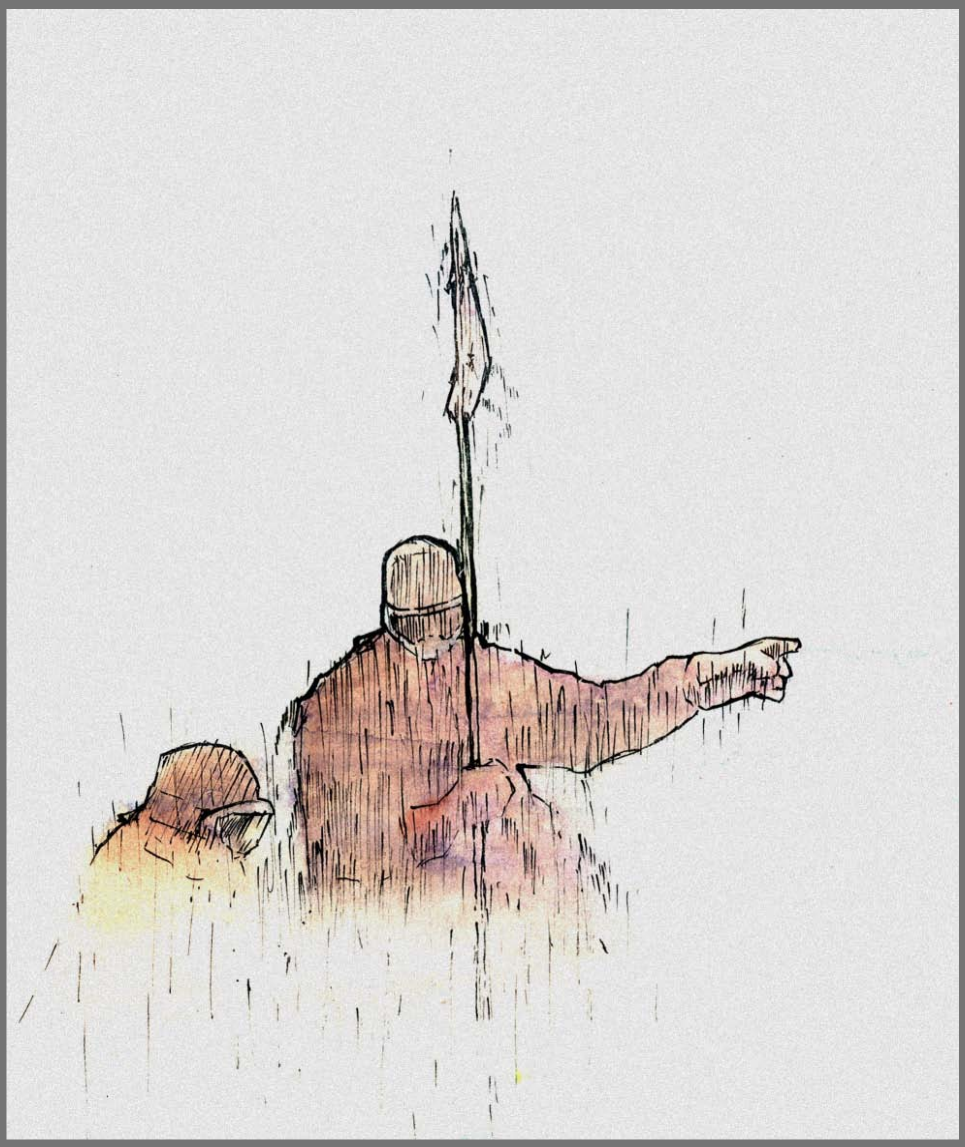
Comunidades microbianas bénticas de zonas polares: Estructura, funcionamiento y ecología

Benthic freshwater communities from polar regions: Structure, function and ecology

Tesis Doctoral/ Doctoral Dissertation



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Universidad Autónoma de Madrid
2011

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Universidad Autónoma de Madrid

Facultad de Ciencias

Departamento de Biología

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Memoria de tesis presentada para optar al grado de Doctor en Ciencias

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A Sofía, Andrea, Totón y Toñín

" As I approached mature years, the action of my heart caused my parents some anxiety; on… examining me… the doctor observed… that it seemed to be beating in rhythm with two short words…South Pole! South Pole! From this moment it became obvious that the words were written there"

The South Polar Times. Robert F. Scott, 1911

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"Cuando viajes hacia Ítaca, pide que el viaje sea largo, lleno de aventuras, lleno de conocimientos"

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Introducción/Introduction

Las cianobacterias en ambientes polares

(Actualmente, este apartado se encuentra en proceso de publicación en la revista Ecosistemas (Asociación Española de Ecología Terrestre) www.revistaecosistemas.net)

"The job of collecting data is rarely done well enough unless it is animated by the prospects of theorethical interpretation, while such interpretation is almost always likely to be the best done by someone who knows the feel of a wire or a rope when a messenger is running down to trip a water bottle…"

G.E Hutchinson 1963. The prospect before us in D.G. Frey (ed). Limnology in North America

RESUMEN

El Phylum Cyanobacteria es uno de los taxones más antiguos de la historia de Tierra. Hay evidencias de que surgieron en el Arcaico y que su actividad biológica ha tenido consecuencias a nivel global. La amplia distribución de comunidades dominadas por cianobacterias en la Criosfera hace que sean una pieza clave en la reconstrucción de la vida microbiana durante las glaciaciones del Precámbrico. Son consideradas los principales fotótrofos de la mayoría de los ecosistemas bénticos polares de agua dulce donde convergen las condiciones adecuadas para que unos pocos organismos, tanto fototróficos como heterotróficos, se asienten formando comunidades con cierta capacidad auto-reguladora. El orden Oscilatoriales es el más representado en estos ecosistemas, probablemente por su amplia tolerancia térmica. Sin embargo, a pesar de no ser especialistas en condiciones de temperaturas cercanas a 0 °C, los niveles tan bajos de depredación que presentan y la flexibilidad y resistencia a condiciones inhóspitas las han promocionado como uno de los organismos más exitosos de la Criosfera.

LAS CIANOBACTERIAS. GENERALIDADES

Las cianobacterias han sido un grupo clave en la historia de la Tierra, son un grupo ampliamente representado tanto en los registros fósiles del Arcaico como del Proterozoico (Schopf 1994, 1993), y han influido tanto en la evolución de los seres vivos como en algunos eventos geológicos con consecuencias a nivel global, como fue la aparición de elevadas concentraciones de oxígeno atmosférico debido a su actividad fotosintética. A grandes rasgos podríamos describir a este grupo de bacterias como organismos fotosintéticos que poseen los fotosistemas I y II localizados en las membranas tilacoidales, excepto en el género *Gloeobacter* que carece de estas estructuras. Sus células normalmente presentan una coloración verde-azulada debido a los pigmentos ficocianina, aloficocianina y clorofila *a*, aunque hay algunas especies que presentan ficoeritrina que confiere un color rojizo a las células. No obstante en algunos grupos taxonómicos también se presentan otras clorofilas (b y d) (Miyashita et al. 1996; Castenholz 2001). Otra característica es que algunos géneros son capaces de fijar nitrógeno atmosférico y presentan diversos cuerpos de almacenamiento para el carbono (gránulos de glucógeno), nitrógeno (cianoficina), fosfato (gránulos de ortofosfato) y la enzima RubisCO (carboxisomas) (Castenholz 2001). Además, la gran variedad de metabolitos secundarios que producen, y sus diversas actividades fisiológicas las dotan de unas características y versatilidad que las convierten en piezas clave de la mayor parte de los ecosistemas polares no marinos.

El registro fósil muestra que las cianobacterias han estado presentes desde el Proterozoico y probablemente ya existiesen en periodos fríos más tempranos (Schopf 2000). No en vano, las cianobacterias son bacterias fotosintéticas oxigénicas Gram-negativas que, de acuerdo con el registro fósil, mantienen su diversidad morfológica desde hace 2000 millones de años (Schopf 2000). Éstas fueron descritas como algas en el s. XVIII y su primera clasificación taxonómica estaba basada en el *Code of Botanical Nomenclature* como se señala en Oren (2004). Así, atendiendo a las clasificaciones botánicas, ha habido dos trabajos de referencia desde entonces. El primero de ellos, a cargo de Geitler (1932),

compilaba la flora de todos los taxones europeos distribuidos en 150 géneros y 1500 especies basadas en su morfología. Más tarde, ha habido numerosas revisiones a cargo de Anagnostidis y Komarek (Anagnostidis and Komarek 1988; Komarek and Anagnostidis 1989) cuya intención ha sido definir géneros más homogéneos entre sí basándose en sus características morfológicas. Aun así, conforme se ha ido profundizando en las características procarióticas de las cianobacterias, en cuanto a su organización ultraestructural y molecular, se ha propuesto que su nomenclatura y taxonomía este regida por caracteres contemplados en el *International Code for Nomenclature of Bacteria* (Stanier et al. 1978) y actualmente el Phylum *Cianobacteria* alberga 5 subsecciones, heredadas de los 5 órdenes de la clasificación botánica, y que están contemplados en Bergey´s Manual of Systematic Bacteriology (Castenholz 2001):

I. Chroococales. Unicelulares o agregados no filamentosos unidos por pared o mucilago; división binaria en uno, dos o tres planos, de firma simétrica o asimétrica, puede reproducirse también por gemación, aparición poco frecuente de células de supervivencia.
II. Pleurocapsales. Poseen células grandes subdivididas en pequeños baeocitos, como forma de resistencia.
III. Oscillatoriales. Filamentosas sin ramificaciones (puede incluir falsas ramificaciones); reproducción por división binaria, sin heterocistos ni acinetos descritos.
IV. Nostocales. Estas son filamentosas no ramificadas y formadoras de heterocistos como formas de resistencia, algunas especies presentan acinetos. Crecimiento similar a Oscillatoriales.
V. Stigonematales. Son filamentosas ramificadas y formadoras de heterocistos.

Aunque esta clasificación está siendo actualmente revisada, hasta la fecha solamente unos pocos nuevos taxones de cianobacterias han sido publicados y validados de acuerdo con los criterios bacteriológicos, esto refleja no sólo las dificultades técnicas que ello conlleva, si no la confusión que subyace de estar incluidas en dos sistemas de nomenclatura distintos (Oren 2004). Actualmente, los estudios taxonómicos sobre cianobacterias se están abordando desde varias perspectivas que combinan estudios genotípicos junto con descripciones morfológicas y análisis fenotípicos, en lo que se denomina la taxonomía polifásica.

LAS CIANOBACTERIAS EN LA CRIOSFERA

En contra de lo que pueda parecer, en torno al 75% de la biosfera es un ambiente frío, es decir que su temperatura media es inferior a 5 °C (Cavicchioli 2006). En ocasiones olvidamos ecosistemas tales como los presentes en el Ártico o la Antártida, o los ecosistemas alpinos de las cordilleras más altas y las profundidades oceánicas o las extensiones de permafrost, los sedimentos marinos, el propio océano y los hielos glaciares, así como las capas altas de la atmósfera (Margesin and Haggblom 2007). En estos hábitats, además de las bajas temperaturas, los ecosistemas están sometidos a otros extremos como los ciclos de congelación-descongelación, variaciones en la radiación recibida (incluida la radiación ultravioleta) y aportes desiguales tanto de nutrientes como de salinidad del medio. Estas características son particulares de ambientes polares (Ártico y Antártida) e incluso alpinos y hacen que *a priori* encontremos una baja biodiversidad. Por ejemplo los animales de cierto tamaño y plantas vasculares son escasos o están ausentes en la mayor parte de los ecosistemas antárticos. Sin embargo, un amplio rango de formas de vida microscópicas entre los que se encuentran protozoos, hongos, bacterias y microalgas son relativamente abundantes. Estos organismos interactúan entre sí para formar dinámicas, y en ocasiones muy estructuradas, comunidades. En este sentido, las cianobacterias son una

pieza clave debido a que son consideradas los principales fotótrofos de la mayoría de estos ecosistemas.

Las comunidades microbianas en ambas regiones polares están dominadas mayoritariamente por cianobacterias del orden Oscillatoriales (Vincent et al. 2000). Como pioneros, estos organismos filamentosos secretan un mucilago orgánico (exopolisacáridos) que dan lugar a estructuras cohesivas y que ofrecen una base muy propicia para la creación de microhabitats en los que se pueden asentar otros microorganismos sucesores con distintas características ecológicas. Estas Oscillatoriales polares tienen una amplia tolerancia térmica (Tang et al. 1997), seguramente reflejo de un origen más templado, y de haberse desarrollado en hábitats muy distintos entre sí, de hecho se pueden encontrar comunidades basadas en cianobacterias en diferentes tipos de hábitats dentro de la Antártida (Vincent 2000b; Priscu et al. 1998).

Datos de paleomagnetismo, junto con otras evidencias geológicas de periodos glaciares en las zonas meridionales del Planeta durante el Proterozoico, han sido interpretadas como consecuencia de cambios en la oblicuidad de la Tierra y de las zonas climáticas en relación a como hoy lo conocemos (Williams et al. 1998). De igual manera estas evidencias han servido para justificar glaciaciones a escala global durante las cuales toda la superficie oceánica y terrestre estaba cubierta de hielo a consecuencia de procesos de enfriamiento acelerados por procesos de retroalimentación (Hoffman et al. 1998; Hoffman 1999; Howard-Williams et al. 1989). Esta conocida y controvertida teoría, *Snowball Earth* (Vincent and Howard-Williams 2000), ha sido apoyada por multitud de modelos climáticos (Jenkins and Smith 1999), pero una de las mayores críticas, en cuanto a la biosfera se refiere, es que un fenómeno glacial de esa magnitud podría haber acabado con la vida (Williams et al. 1998). Dejando aparte argumentos a favor y en contra de esta hipótesis, si que podemos observar que tanto en el Ártico como en la Antártida existen comunidades microbianas que se desarrollan en íntima relación con el hielo, sugiriendo qué en episodios glaciares de escala global estas comunidades basadas en cianobacterias

podrían haber tenido una mayor importancia, incluso haber constituido refugios para la supervivencia, desarrollo y evolución (Margulis and Sagan 1997) de una gran variedad de organismos, incluidos eucariotas pluricelulares que posteriormente radiarían con la llegada de periodos más cálidos (Vincent et al. 2000). La distribución de estas comunidades dominadas por cianobacterias en los ecosistemas fríos de la Tierra, hacen que sean una pieza clave en la reconstrucción de la vida microbiana y su diversificación en los primeros estadios de la historia de la Tierra (Vincent et al. 2004). Así, se presentan como muy buenos modelos, e incluso análogos, de los biotopos que se pudieron dar durante las glaciaciones del Precámbrico. Hace unos 200-160 millones de años, la Antártida junto con Australia, América del Norte y América del Sur, India y Nueva Zelanda formaban el supercontinente Gondwana. Más tarde, con la progresiva separación de Australia, unos 45-50 millones de años desde la actualidad, comenzó la ruptura de este supercontinente, que continuó con la separación de América del Sur, hace 30 millones de años, empujando a la Antártida a la zona sur de la Tierra, donde se encuentra actualmente (Figura 1). Este último desplazamiento dio lugar a la formación del Estrecho de Drake (Figura 1), permitiendo que se creara una corriente circumpolar tanto de bajas presiones como de aguas de distinta densidad. Este aislamiento llevó al enfriamiento de todo el Continente Austral a niveles parecidos a los que conocemos en la actualidad. Sin embargo, sólo podemos remontarnos poco más de un siglo atrás, a la época de las grandes expediciones, para encontrar los primeros registros sobre cianobacterias en la Antártida. James Murray en 1910, durante la expedición de Ernest Shackelton a Ross Island entre 1907-09, mientras hacían algunos trabajos de perforación del hielo que cubría un lago cercano a su campamento, dejó constancia en su diario de una de estas comunidades: "*that on careful thawing released a multitude of living things for study*" (Vincent and Quesada 2011). La Antártida está cubierta en un 99.6% por una capa de hielo permanente (*inlandsis*) de un grosor medio de unos 2 km, con máximos de hasta 4 km, que dejan al descubierto tan sólo las cumbres rocosas de algunas montañas que permanecen libres de

hielo (*nunataks*). Éstos, junto a las zonas costeras son ese pequeño porcentaje del continente que permanece libre de hielo y en el que se dan hábitats menos severos en los que se asientan multitud de seres vivos (Hughes et al. 2006).

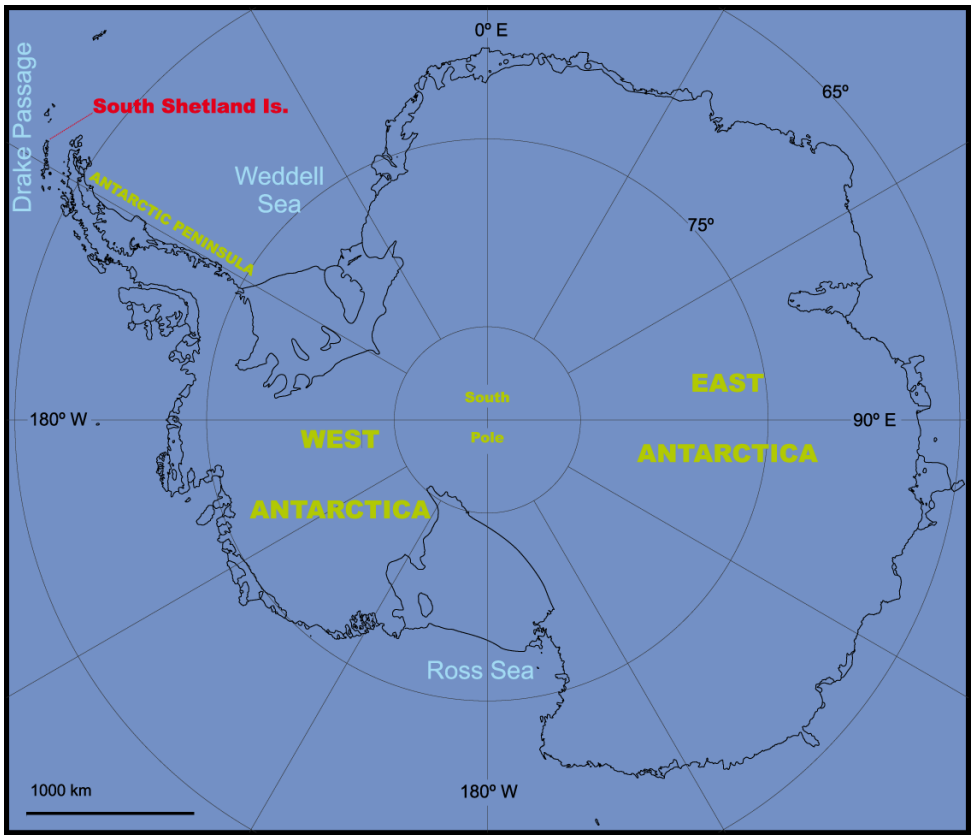

Figura 1. Mapa de la Antártida.

Desde un punto de vista ecológico, como propone Vincent (2009), la mayoría de las cianobacterias de la biosfera se pueden clasificar en tres grupos funcionales: *picocianobacteria*, *formadoras de blooms* y *formadores de tapetes*. Las *picocianobacterias* son muy comunes en cuerpos de agua dulce oligotróficos muy característicos de zonas de latitudes altas. Por el contrario, éstas están poco representadas en los océanos que circundan estas zonas polares (Vincent 2000a). Las *cianobacterias formadoras de blooms*, ausentes en la mayor parte de los ecosistemas acuáticos polares, tanto oceánicos como de agua dulce, si se han descrito en aguas de zonas subárticas. Las predicciones de cambio global hacen pensar que este tipo de cianobacterias incrementará su presencia en zonas

de influencia polar. Por último, estarían las *cianobacterias formadoras de tapetes,* con diferencia el grupo con más éxito en las regiones polares, cuya característica principal es que acumulan grandes cantidades de biomasa en determinadas regiones, y se han considerado como las únicas formas de vida capaces de permanecer en los ambientes polares más extremos de la Tierra (Verleyen et al. 2010).

Habitualmente se considera que la biodiversidad disminuye con el aumento progresivo de la latitud, llegando a registros mínimos en las zonas polares más extremas. Este patrón se atribuye a un gradiente de temperatura, humedad y duración de la estación estival (Smith 1994; Kappen 2004; Convey 2001). De hecho, los mayores índices de biodiversidad terrestre de todo el Continente Antártico se dan en los archipiélagos antárticos y subantárticos que se encuentran en la periferia del Continente (Convey and Stevens 2007). Sólo en determinados "oasis" de vida dentro del Continente, como pueden ser lagos, lagos subglaciares o *nunataks*, convergen las condiciones adecuadas para que unos pocos organismos se asienten formando comunidades con cierta capacidad auto-reguladora que permiten su establecimiento y desarrollo, suavizando el efecto de los factores físicos dominantes (Camacho 2006). Sin embargo, este patrón que relaciona la pérdida de biodiversidad con el aumento de latitud debe ser considerado con cuidado, ya que proviene de la aplicación directa de observaciones y patrones que se dan en organismos más complejos y su aplicación directa a la diversidad microbiana y de cianobacterias no tiene por qué ser correcta. De hecho recientemente, en la Antártida se han descrito altísimas diversidades tanto de procariotas (Tindall 2004) como de eucariotas (Lawley et al. 2004), así como de virus (Lopez-Bueno et al. 2009), incluso mayores que en zonas más meridionales del Planeta.

ADAPTACIONES DE LAS CIANOBACTERIAS A LAS CONDICIONES POLARES

Además de los atributos fisiológicos propios de cada taxón, el éxito en la colonización en ambientes polares inevitablemente exige tolerancia a bajas temperaturas. La mayoría de los organismos procariontes parecen ser psicrotolerantes, capaces de crecer a temperaturas cercanas a 0 °C, pero con una temperatura óptima de crecimiento de unos 20 °C (Morita 1975). Sin embargo, hay relativamente pocos "verdaderos" procariontes psicrófilos en la Antártida, incapaces de crecer a temperaturas superiores a 15 °C, incluso en aquellos ambientes donde las temperaturas medias del aire están cercanas a 0 °C en verano. La psicrofília, entendida como adaptaciones de determinadas enzimas o rutas enzimáticas con rendimientos óptimos a temperaturas menores de 10ºC paradójicamente no aporta una ventaja ecológica para los organismos en determinados ambientes polares. La fluctuación diaria de temperatura durante los periodos estivales (Figura 2) implica que, organismos capaces de soportar rangos de temperatura mayores que los óptimos de un psicrófilo *sensu estricto*, se vean favorecidos (Velázquez et al. 2011).

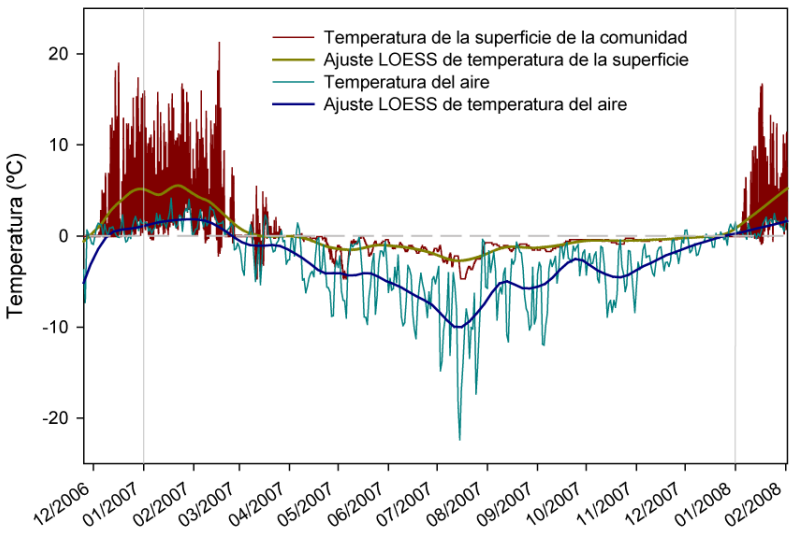

Figura 2. Régimen de temperaturas de la superficie de un tapete de cianobacterias comparada con la temperatura del aire. Los datos fueron tomados en la Península Byers (Isla Livingston, South Shetland Islands).

Nuestros resultados han mostrado como los tapetes de cianobacterias tienen su óptimo metabólico a temperaturas muy superiores a las que habitualmente están

expuestos en los ambientes polares (Velázquez et al. 2011). Sin embargo, las comunidades algales eucariontes que se encuentran formando biofilms en estos ambientes muestran una adaptación muy intensa a estas condiciones climáticas (Velázquez et al. 2011). De esta manera, mientras que los tapetes de cianobacterias presentan una actividad fotosintética muy reducida entre 0 y 10 °C las algas verdes tienen su máximo en temperaturas cercanas al punto de congelación (Figura 3).

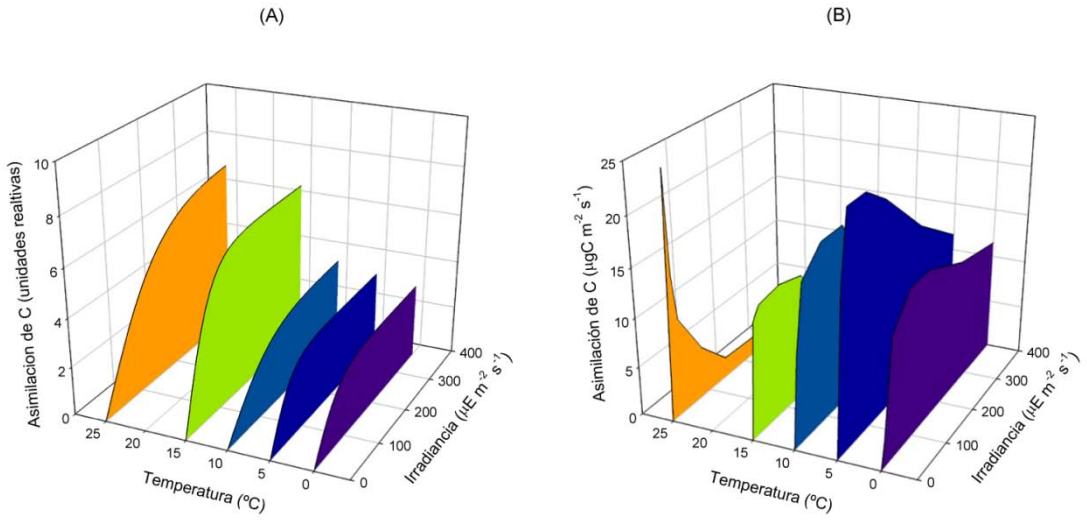

Figura 3. Ensayos de fotosíntesis a distintas irradiancias y temperaturas de un tapete bentónico cianobacteriano (A) y de una comunidad criosestónica dominada por algas verdes (B) en la Península Byers (Isla Livingston, South Shetland Islands).

Ciertamente, las tasas de producción de las cianobacterias son modestas en las condiciones que habitualmente aparecen en estos ecosistemas, pero a largo plazo su resistencia y flexibilidad las hace dominar todos los ecosistemas someros de las zonas polares, en lo que se ha dado por llamar la *estrategia del liquen* (Quesada and Vincent 2011).

Quizás parte de éxito de las cianobacterias en estos ecosistemas radica en la estructura que adoptan comunidades, que consisten en una tupida malla habitualmente formada por la Oscillatorial *Leptolyngbya spp.*, que, con un diámetro a veces inferior a 1 µm (de los Rios et al. 2004), conforman un ambiente donde las condiciones pueden ser ligeramente más benignas y donde se instalan otros muchos organismos. Estos ecosistemas basan su

estabilidad en la ausencia de depredadores efectivos, que puedan acabar con la escasa aportación de C y energía debida a la fotosíntesis de las cianobacterias. De cualquier manera se estima que la limitación en estos ecosistemas radica, realmente, en la presencia de agua líquida sólo durante unas semanas al año, lo que probablemente limita el desarrollo de una fauna capaz de consumir los recursos elaborados por las cianobacterias. Dicho de otra manera, los tapetes de cianobacterias de zonas polares con climas menos severos cuentan con un elenco de formas y organismos, compuesto principalmente por virus, bacterias, hongos, rotíferos, nematodos y tardígrados que permiten un reciclado continuo de la materia y de la energía acumulada por los productores primarios, fundamentalmente las cianobacterias (Velázquez, 2011). Al avanzar en latitud a ambientes polares más severos, estos organismos se reducen en abundancia y relevancia y el reciclaje tiene lugar de una manera incompleta lo que conduce a acumulaciones de grandes cantidades de materia orgánica sin degradar (Fernández-Valiente et al. 2001).

Otro aspecto ampliamente controvertido es la presencia de cianobacterias endémicas de la Antártida o en sentido más amplio de las zonas polares o de la Criosfera. Varios autores (Taton et al. 2003 y las referencias de su interior) han sugerido la presencia de cianobacterias endémicas de la Antártida basándose en secuencias genéticas ampliamente distribuidas en la Antártida pero aparentemente ausentes en otras latitudes. Sin embargo, según se completan los bancos de datos genéticos, estos endemismos aparecen en otros lugares fuera de su teórica circunscripción antártica (Jungblut et al. 2010; Strunecky et al. 2010). Aún así, muchos de estos supuestos endemismos antárticos aparecen únicamente en ambientes extremos aunque no necesariamente relacionados con el hielo o con el frío, como son algunas cianobacterias consideradas “antárticas” y que han sido recientemente descubiertas en lagos someros salinos del Desierto de Atacama (Chile) (Dorador et al. 2009). De esta manera podríamos considerar la existencia de un grupo de taxones de cianobacterias que pueden sobrevivir en condiciones extremas y que estas se distribuyen en aquellos lugares adecuados para su lento crecimiento.

En definitiva las cianobacterias son los organismos que dominan los ecosistemas no marinos en ambas regiones polares. Esta dominancia no se basa en una especial adaptación de estos organismos a unas condiciones excepcionalmente rigurosas, sino más bien en una flexibilidad y en una resistencia a estas condiciones inhóspitas que las permite subsistir en la Criosfera.

Agradecimientos: Los autores muestran su gratitud a todo el grupo de científicos y técnicos que han trabajado en el Proyecto LIMNOPOLAR durante su trayectoria de más de 10 años. Este trabajo ha sido realizado con financiación del Ministerio de Ciencia e Innovación a través de los proyectos financiados con las siguientes referencias: CGL2005-06549 y POL2006-06635 entre otros. David Velázquez ha recibido una beca del Ministerio de Ciencia e Innovación para el desarrollo de su Tesis Doctoral. Agradecemos en particular el apoyo y consejo de D. Manuel Bañón. Este trabajo no se podría haber realizado sin el apoyo logístico de la UTM y la ayuda incondicional del BIO Las Palmas de la Armada Española.

Hipótesis y objetivos/Hypothesis and aims

El presente trabajo profundiza en el estudio de las comunidades bénticas microbianas que se desarrollan en las zonas polares desde una perspectiva ecofisiológica y ecológica. Para ello, se han visitado ambas áreas, Ártico y Antártida marítima, desarrollando trabajos de fisiología, taxonomía y ecología con abordajes principalmente *in situ*.

Debido a que estas comunidades bénticas se distribuyen por ambas zonas polares, quisimos determinar los factores ambientales que determinaban su composición, presencia y distribución a lo largo de un gradiente latitudinal polar. La hipótesis de partida era que no existía ningún patrón que relacionase la composición de productores primarios (refiriéndonos únicamente a los grupos de cianobacterias y algas verdes) de los tapetes microbianos con la latitud y que los factores fisicoquímicos no determinarían su composición taxonómica. Por el contrario, serían tanto la historia ecológica de cada comunidad, así como las características del medio donde se desarrollen, uno de los factores más importantes que determinen su presencia y composición en ambas zonas polares. Para el desarrollo de estas ideas se trabajó con distintas comunidades bénticas de sendas áreas polares, registrando una gran variedad de factores ambientales y caracterizando la taxonomía de cada tapete, relacionando todas ellas mediante un análisis estadístico multivariante.

Así, se propuso ver cómo era la dinámica de estas comunidades en esos primeros estadios de cada estación estival y si esto podría tener alguna influencia en estadios posteriores. La hipótesis que se planteó, en cuanto a la dinámica de estas comunidades bénticas al comienzo de cada estación estival, era que el régimen de temperaturas es un factor clave en el desarrollo de estas comunidades y que determina tanto su composición, mediante sucesión, como su actividad fisiológica. Así, pequeños cambios de temperatura

puedrian dar lugar a variaciones en la cantidad de biomasa acumulada y cambios en los ciclos biogeoquímicos que se dan entre los organismos de estas comunidades. La aproximación elegida fue extraer e incubar una comunidad béntica y ver así su desarrollo durante un breve periodo de tiempo (varias semanas) mediante incubaciones en cámaras climáticas a distinta temperatura. De forma paralela, se fue siguiendo el desarrollo de la comunidad en su estado natural para poder comparar los tratamientos *ex situ* con el desarrollo natural de la misma comunidad.

Entonces, tomando la temperatura como uno de los factores principales de terminantes de la sucesión ecológica y composición de estas comunidades, se planteó ver sus efectos a corto plazo (horas) en comunidades bénticas con distinta composición taxonómica. Los organismos de estas comunidades presentan dos tipos de estrategias en relación a sus óptimos de temperatura: psicrófilos (aquellos cuya temperatura optima de desarrollo está por debajo de 15 °C) y psicrótrofos (aquellos que pueden sobrevivir en zonas frías aunque su óptimo se encuentra a 15 °C o por encima). La hipótesis principal que se quiso comprobar fue si el carácter psicrófilo o psicrótrofo de los productores primarios de las comunidades bénticas modula los ciclos tanto del C como del N y su sucesión ecológica.

Para caracterizar estas comunidades se hicieron experimentos de asimilación de C y N a distintas temperaturas, evaluando el efecto a corto plazo de ésta sobre las comunidades. Para ello se eligieron dos comunidades: una compuesta principalmente por cianobacterias y la otra por algas verdes, ya que *a priori* ambas comunidades presentan distintos óptimos de temperatura para sus actividades metabólicas. Lejos de establecer paralelismos con el cambio climático que afecta a estas zonas polares, esta aproximación permitió caracterizar su estado fisiológico y dio pie al planteamiento de las sucesivas las hipótesis que se desarrollarían a lo largo del presente trabajo, ya que a pesar de ser las

cianobacterias los elementos más conspicuos de estas comunidades, alguna de ellas parecía no estar en su óptimo de temperatura.

Por último, después de haber caracterizado el comportamiento en función de la temperatura y debido a que estas comunidades constituyen la mayor parte de la biomasa de las zonas polares se realizó un seguimiento estacional cubriendo la mayor parte del periodo estival, identificado como el de actividad máxima de estas comunidades. La hipótesis principal que planteada considera que los aportes de C inorgánico, así como otros factores fisicoquímicos, no limitan el aumento en biomasa ni la productividad de estas comunidades, si no que las relaciones tróficas entre los distintos grupos de organismos que la conformaban, así como los procesos de reciclado, tienen un peso mayor del que hasta la fecha se creía. Para comprobar dicha hipótesis se midieron distintas actividades fisiológicas a lo largo de la estación estival y todo ello cristalizó en un modelo trófico implementado por un análisis de la red establecida en este ecosistema y de las características globales que de la comunidad emanan (Ecological Network Analysis, ENA).

Capítulo 1/Chapter 1

Response of microbial mat communities to the physical and chemical environment of polar regions.

"Science and exploration have never been at variance; rather, the desire for the pure elements of natural revelation laid at the source of that unquenchable power- the "love of adventure"

Douglas A. Mawson. The home of the blizzard.

ABSTRACT

Polar landscapes are dominated by ice and snow, yet paradoxically it is liquid water that drives ecosystem processes in these desert systems. In the extreme Arctic and Antarctic climate, the abundance of liquid water is determined by delicate balances of radiation and temperature and their effects on the ice-water phase change. As such, ecosystem processes are sensitive to climate variability (natural or anthropogenic, spatial or temporal) and community composition. Cyanobacteria and green algae diversity were investigated *in vivo* in polar freshwater ecosystems during both summer seasons within the same year. In total, 29 taxa were found, and a set of environmental variables were correlated to this diverse primary community's composition by a multivariate approach. The results pointed out the ecological and taxonomical differences in cyanobacterial and green algae composition of both polar regions. Each community was supported by a cyanobacterial matrix, but Arctic communities presented higher abundance of green algae than those from Antarctic ponds. Also there were not any single environmental variable explaining such differences, but a selection of sub- sets of variables may act as proper proxies to communities´ composition attending to Cyanobacteria and Chlorophyte assemblages.

INTRODUCTION

Terrestrial ecosystems in Antarctica include a great variety of habitats from extremely cold and dry ice-free areas of the Continent to the comparatively warmer sub-Antarctic. Maritime Antarctic comprises the western side of the Antarctic Peninsula and islands of the Scotia Arc (South Shetland, South Orkney and South Sandwich Islands) at 55–68 °S. Whereas, the High Canadian Arctic consists of the northern fingers of Ellesmere Island, in the region of 80–85 °N. A large proportion of the relatively small ice-free area of polar regions consists of cold deserts, where microbial community development is mainly restricted to three types of habitat: endolithic communities inside rocks, freshwater communities in transient water bodies, and hypersaline ice-covered lakes (Wynn-Williams 1990).

In ice-free areas from polar regions in summer, shallow low productivity lakes and meltwater streams are locally abundant. However, smaller inland water bodies freeze solid or dry up for an extended period every year, thus providing rather unfavourable conditions for the biota. As larger metazoans are often rare or absent (in particular in the Antarctic) (Maslen and Convey 2006), the aquatic communities are dominated by microscopic organisms, mainly dominated by cyanobacterial and green algae, that play a major role in the biological transfer of energy and matter (Fogg 1998; Vincent 2000b). Previous work on polar cyanobacteria using both morphological and molecular methods in the polar regions, has mostly been performed in the Antarctic, where cosmopolitan and endemic taxa are reported (Komarek and Anagnostidis 1999; Taton et al. 2003; Taton et al. 2006; Jungblut et al. 2010; Jungblut et al. 2005; Comte et al. 2007). By comparison, little is known about Arctic cyanobacteria, which although inhabiting a similar environment, are potentially more connected to temperate latitudes than Antarctic cyanobacteria, which are relatively isolated by the Southern Ocean. Benthic microbial mat communities dominated by cyanobacteria are widespread throughout the meltwater environments, including lakes, ponds and streams in the Arctic (Vincent et al. 2004; Mueller et al. 2005) and

Antarctic (Howard-Williams and Vincent 1989; Zakhia et al. 2008; Howard-Williams and Hawes 2007) regions. These communities dominate the total biomass and the biological productivity in these meltwater environments due to their capability to grow on the polar environments (Vincent 2000).

In most shallow polar freshwater ecosystems, fast growing phytoplankton occurs in sparse concentrations in the water column. This contrasts with the benthic communities in such waters, which often form well-developed, perennial mats and films (Bonilla et al. 2009). The benthic communities may be up to several millimeters thick and are often dominated by cyanobacteria (Sabbe et al. 2004; Vincent 2000a; Hodgson et al. 2001). These interacting algal communities differ in composition, growth rates, loss processes, tolerance to nutritional stress and ability to optimize light use (Vézina and Vincent 1997; Bonilla et al. 2005). Such differences are likely to affect the algal sensitivity to various stressors, including climate change. Predicted future environmental changes in the polar regions include increased air temperatures, decreased duration of snow and ice cover, increased evaporation, changes in colored dissolved organic matter (CDOM) and increases in exposure to ultraviolet radiation (UVR), and these will likely have broad impacts on polar freshwater ecosystems (Vincent and Laybourn-Parry 2008 ; Smol and Douglas 2007; Wrona et al. 2006). Abundant data indicate that the growth of photosynthetic biota as well as large scale ecosystem primary production is frequently limited by supplies of some nutrients, as P and N, in freshwater environments (Elser and Goldman 1990), internal food web structure (Elser et al. 1988) and many other processes can all affect the absolute and relative supplies of nutrients in lakes, ponds and streams and hence their conditions. Indeed, elevated inputs of these nutrients have been implicated worldwide in massive changes in biological diversity (Smith et al. 1999).

Here, it is reported the results of a meta-analysis that compiled and analyzed data from field experiments evaluating the status and diversity of primary producers. Our goal was to determine if patterns of autotrophic assemblages (cyanobacteria and green algae)

and the relationships amongst variables differ across latitudinal changes in both polar areas. Hence, biodiversity of green algae and cyanobacteria of the freshwater communities were investigated in the maritime Antarctic and the High Canadian Arctic during one summer expedition to each location within the same year (2009). Apart from their physiological adaptation to broad ranges of temperature and other environmental variables, these communities have been of interest to microbial ecologists because of their biogeography. A long-standing theory of microbial distribution is that 'everything is everywhere' and that the local environment selects for a particular microbial flora that is globally distributed. Hence, the present study aims to highlight the biogeography patterns of those sorts of communities and some of the organisms (as green algae and cyanobacteria) living within. Most of the previous approaches have been focused on biogeographical patterns of different taxa (Jungblut et al. 2010; Taton et al. 2006; Taton et al. 2003; Strunecky et al. 2010), but our aim is to unravel the biogeographical patterns of different polar assemblages from an ecological perspective instead of a taxonomical focus.

MATERIALS AND METHODS:

Study sites

Experiments were performed in four sites, two at Canadian Arctic and two in Antarctica, during boreal summer of 2009 and along the 2008-09 austral summer season, respectively. The main Arctic area, with 6 microbial communities surveyed, was Ward Hunt Island and surroundings (83º N) and 2 more communities at Cornwallis Island (74ºN). The Antarctic communities assayed were collected from Ares and Mars Oases (Alexander Island, Antarctic Peninsula; 72ºS) and at Byers Peninsula (Livingston Island, South Shetland Islands; 63ºS) (figure 1) (table 1).

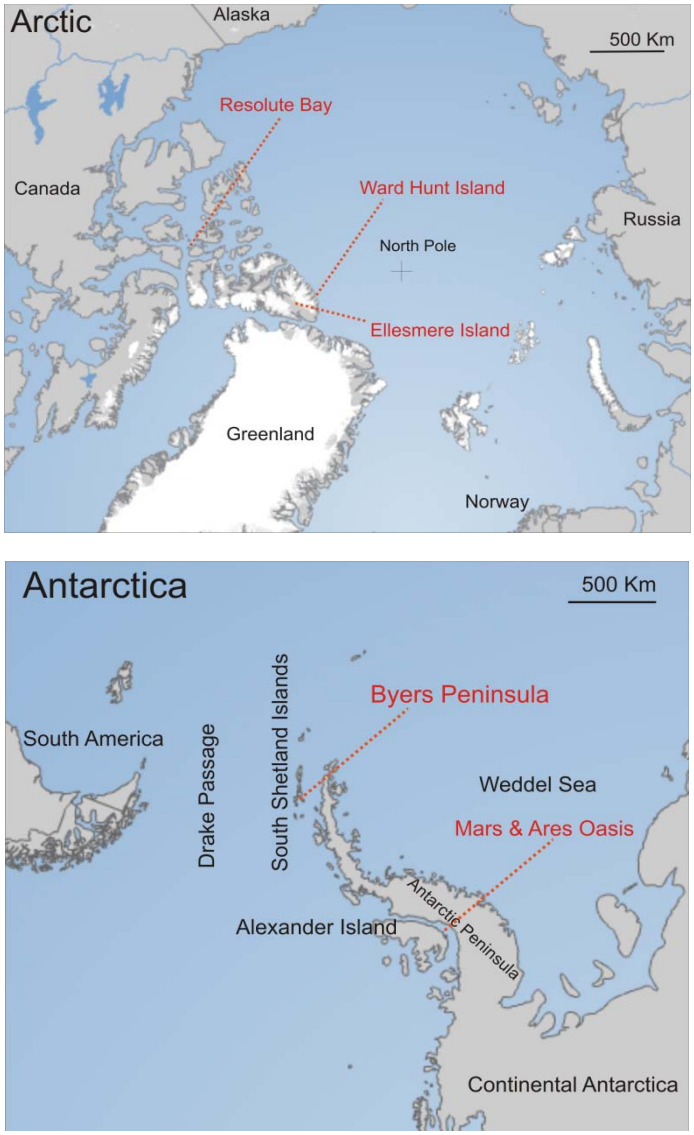

Figure 1. Sampling site location at both polar regions.

Arctic Sites

Figure 2. Composite of pictures from High Canadian Arctic assayed sites. (A) W1, also known as Antoniades Pond. (B) W2 at Ward Hunt Island. (C) W3 at Ellesmere Island. (D) detailed picture of W3. (E) stream input flow to Lake A where W4 was sited. (F) W5. (G) detailed picture of W5. (H) Ward Hunt Lake covered still by ice cap, at the background northern limits of Ellesmere Island. (I) detailed picture of the PvsI assay set-up. (J) Ice moat over Meretta Lake, at the background the installations of Polar Continental Shelf Project (PCSP) of Canadian Government. (K) R2 pond at the background of the picture.

1. Ellesmere Island, Nunavut

The most northern sampling ponds were sited at Ward Hunt Island and Ellesmere Island. Those are located within *Quttinirpaaq* National Park ("top of the world" in Inuktitut); it represents Canada's Eastern High Arctic Glacier Natural Region and a mountainous portion of the Northern Arctic Marine Region. Ward Hunt Island is located at the northern end of the Ellesmere Island, which itself is the northern extremity of the Arctic Archipelago. The 900 m thick icefields are a true remnant of the last continental glaciers that covered much of North America during the last Ice Age some 10 000 years ago. Being as far north as it is, Ward Hunt Island has wintry weather conditions even in the summer. July is the only month where temperatures reach into the 0 °C to 7 °C range. Summer also brings several weeks of continuous daylight. Winters are long and harsh, summers cool and brief, with cold dry winds and very light snowfalls. The area is essentially a polar desert, though thermal oases, warm and moist enough to support life. Only about 60 mm of precipitation falls here annually (*Parks Canada website*).

Samples from Ellesmere Island in Quttinirpaaq National Park, Canadian High Arctic, were taken between 3 and 20 July 2009 from the following sites (figure 2):

- **W1 (Antoniades Pond)** (figure 2a), with an area of approximately 300 m^2, contained thick mucilaginous orange pigmented microbial mats that covered the littoral zone.

- **W2** (figure 2b) was brownish orange and well developed, sited 200 m W of our camp in an small water flow heading to Quttinirpaaq lagoon, the northern limit of Ward Hunt Island. That has its source at the northern shore of Ward Hunt Lake. Mat was growing under around 2 cm of water and it had evident Nostoc spp. colonies and some green filamentous algae growing on top of the mat. The stream was surrounded by some grasses.

- **W3** (figure 2c and 1d). The pond was elliptical about 3 by 7 m. A well developed microbial mat covered the whole pond bed. It was based on a *Leptolyngbya* spp. structure with abundant Nostoc-like colonies growing on top. The mat brownish colour is almost white on surface due to carbonate deposits over its surface. The pond was surrounded by many moss patches.

- **W4** (figure 2e) was a backwater input to Lake A (Tomkins et al. 2009), with 1-2 m wide and few centimetres deep, enough to maintain a sheet of Nostoc- based community.

- **W5** (figure 2f and 1g). This mat covered a 5 by 7m area and grew up on a wide network of streams and pools running from a moraine glacier. Many macroscopic Nostoc-like colonies are conspicuous on the surface of the microbial mat.

- **W6** (figure 2h and 1i). The lake has a maximum depth of 5.5 m and a total area of 0.37 km^2, and is the most northern lake of North America (Villeneuve et al. 2001) the ice-free littoral zones are completely covered by cohesive microbial mats.

2. Cornwallis Island, Nunavut

The area around the hamlet of Resolute Bay (also known by the Inuktitut name *Qausuittuq*) is underlain by Silurian limestone of the Read Bay Formation (Douglas and Smol 2000). The mean daily temperature in summer is above 0 ºC (e.g. July average of 2 ºC), but temperatures can often drop below -30 ºC in winter (e.g. January average of -33 ºC (Maxwell 1980)). Annual precipitation, corrected for snow gauge undercatch, is about 200 mm (Woo et al., 1983). The area is classified as a polar desert. Although snow typically melts in late June, lakes such as Meretta may remain ice covered all summer by a central float of snow and ice, or may thaw totally in late July or more typically in August. By late August or early September, winter conditions begin to return, and so the open water season is greatly truncated compared to temperate lake systems (Douglas and Smol 2000).

At Cornwallis Island two benthic communities were surveyed, those were at PCSP buildings (Polar Continental Shelf Project) surroundings at Resolute Bay.

- **R1** (figure 2j). This mat was growing on the bed of Meretta Lake (72º 41,750 N, 94º 59.58 W) and samples were taken on the northern shore. Much research has been carried out on Meretta Lake: e.g. Schindle et al. (1974). Briefly, Meretta Lake has two basins. The northern basin has a surface area of 20.0 ha, a maximum depth of 9.0 m, and a mean depth of 3.2 m. Meretta Lake has received raw sewage from the Department of Transport Airport Base since the construction of the so-called 'North Base' in 1949.

- **R2** (figure 2k). The benthic microbial community grows in a shallow pond, covering an area of about 50 m by 25 m north of Meretta lake about 200 m west of the PCSP facilities. Due to the direction of prevailing winds in the area, this gets a significant amount of plastic waste,. The benthic community seems covers the pond bed.

Antarctic Sites

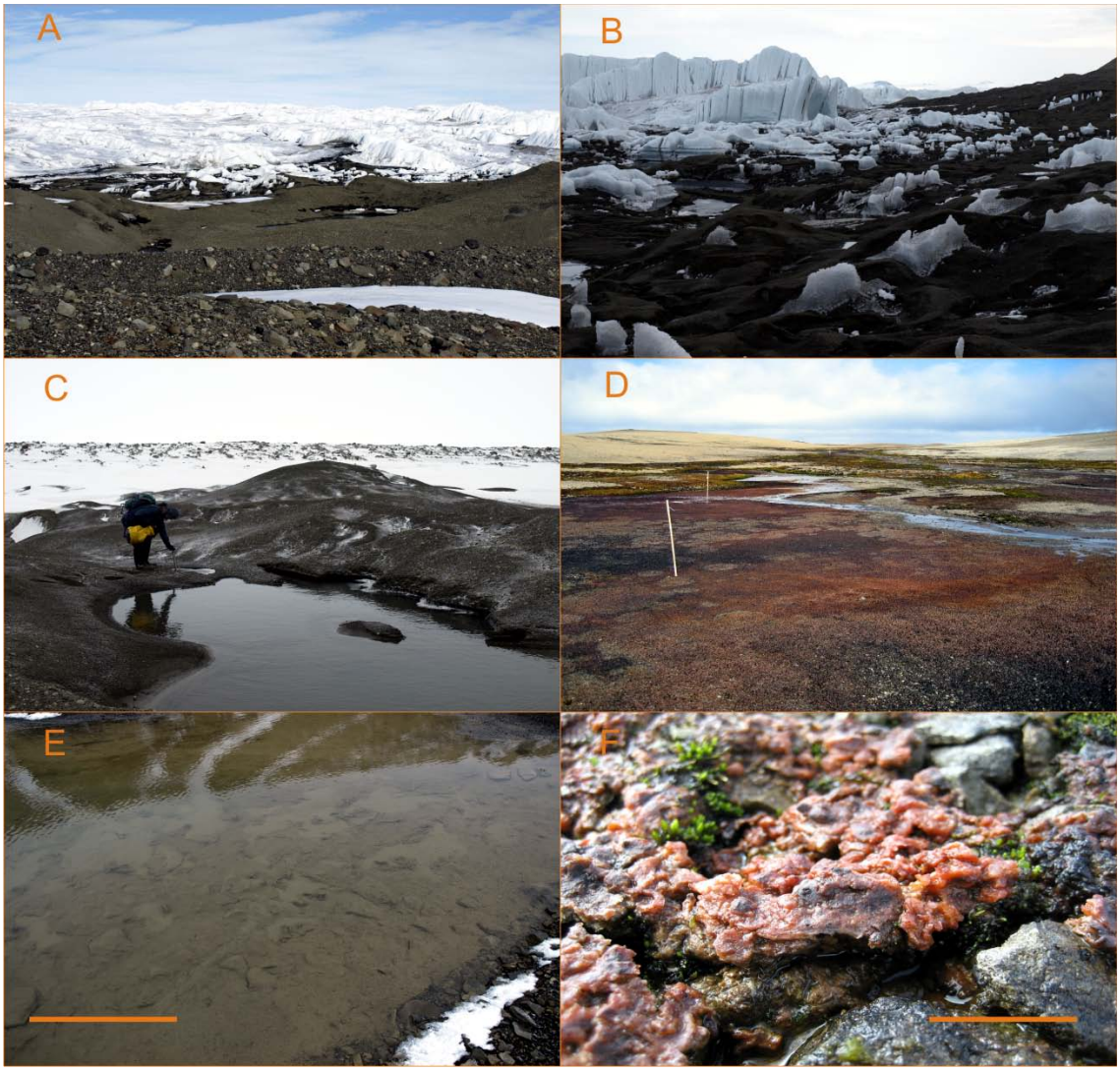

Figure 3. Composite of pictures from the Antarctic assayed sites at Alexander Island and Livingston Island. (A) panoramic landscape of Ares Oasis. (B) landscape from Mars Oasis. (C) works of ponds surveying at Ares Oasis, west limit of A3. (D) Byers Peninsula landscape where SW mat was sited. Wood pools length 1.5m. (E) detailed picture of A3 mat. Bar means 10cm. (F) Detailed picture of SW mat. Bar means 1cm.

1. Alexander Island

The study areas were located in the south-eastern part of Alexander Island. It is the largest island located off the Antarctic Peninsula from which it is separated by George VI Sound that is 500-600 m deep at its northern end. This tectonic trench is occupied by an ice shelf 500 km long, 20-60 km wide and 100-500m thick, that is fed by outlet glaciers flowing from the Palmer Land ice cap (Sugden and Clapperton 1981). In the Ares and Mars oasis

areas, a series of ice shelf moraines 2 to 10 m high run parallel to the pressure ridges of George VI Ice Shelf that occur at 50 m asl. these belong to a 120 km long ice shelf morainic complex running along the eastern flank of Alexander Island form Ablation Point to Two Steps Cliffs (Sugden and Clapperton 1981) (figure 3). Mars oasis (35 m asl) is occupied by glacial and glacifluvial material, ponds and moss banks.

The samples from Mars Oasis and Ares Oasis in Alexander Island, Antarctic Peninsula, were taken between 10 and 11 December 2008 from the following sites:

1a. Mars Oasis

- **M1** (Worland Pond) (figure 3), close to the apple hut installed by BAS. The Pond was approximately 50-60m by 10-15m with a maximum depth of 40 cm. This consists of four irregular lobes and the whole bed was covered with a well developed mat. It has an area with well developed colonies of Nostoc spp.

- **M2** (figure 3) is close to M1 but not connected to it. The pond was differentiated in two lobes and had an extension about 45m by 20m. There were abundant microbial crusts at some of its banks. The orange microbial mat is very well differentiated into two layers.

1b. Ares Oasis

- **A1** (figure 3). The pond bed was completely covered by a microbial mat. It seems to be a residual pond from another 2 or 3 times larger. On its shores old microbial crusts, even at 50cm above the current water-mirror, were found. This water level loss remains unexplained, although it could be caused by evaporation or ice sinks. The mat was thick and orange coloured following the surface relief underneath.

- **A2** (figure 3). The pond sited at a lower level than the A1 but close to it. However, it did not seem to be connected by surface flow. It was partially covered by ice. It

appears, too, the remains of a larger pond that has broken its embankment resulting in small ponds. There were old microbial mats in areas higher than the current edge of the pond. The remaining ponds were connected by small intermittent streams and appear to be colonized by at least different colour and texture mats. The microbial mat had arbuscular shape and is bright orange.

- **A3** (figure 3c and 2e) was sited below A2 and most of the water comes from it, but it also has another surface water input. The mat was very cohesive and slim and covered by sediments from A2, probably.

2. Byers Peninsula, Livingston Island

Another site form Antarctica was also included on the analysis. That is **SW** (figure 3d and 2f) which is sited at Byers Peninsula. This is the largest ice-free area in Maritime Antarctica and supports very abundant and diverse aquatic ecosystems (Toro et al. 2007; Fernández-Valiente et al. 2007). The area is located at the west end of Livingston Island (62º 34'35" - 62°40'35" S and 60°54'14" - 61°13'07" W) (figure 1). The peninsula, 60.6 km^2 in area, is largely free of ice and snow cover during almost the entire austral summer season (December to March). The low-lying areas of the peninsula favour the retention of surface water and include temporary waterlogged areas, lakes and pools (López-Martínez et al. 1996). The microbial mat studied here grew on the plateau of the Peninsula (Velázquez et al. 2011) (figure 3).

- **SW** (figure 3d and 2f). This microbial mat was 3-4 mm in thickness and had a rugged surface that shows a purple-greenish color. The mat occurred in a semi-permanent shallow pond. This wetland area covers a surface area about 20 ha.

Physiochemical analysis

In the Arctic, water temperature, pH and conductivity were determined at each site using a portable instrument (pH/Con 10 series; Oakton Instruments, Vernon Hills, IL, USA). Water

samples for nutrient analysis were collected from just above the microbial mats, in acid-washed bottles, and stored at 4ºC until analysis. Total nitrogen and total phosphorous were determined by standard methods (Strainton et al. 1977); QuikChem 10-107-06-2-K) at Institute National de la Recherche Scientifique (Quebec City, QC, Canada). In the Antarctic communities, environmental variables, pH, temperature conductivity were determined by a portable multiparametric probe (YSI 6920).

In the laboratory, cores from all the communities were weighed and dried at 60ºC for 24 hours. After grinding, the isotopic enrichment of samples relative to non-treated controls was determined using an IRMS Micromass-Isochrom mass spectrometer. The particulate organic carbon (POC) and nitrogen (PON) content of both communities were measured from the dried samples by using an Elemental Analyser LECO CHNS-932. Due to carbonate deposits in the Arctic samples, those were previously treated by HCl 1N to eliminate the inorganic carbon on the communities.

Photosynthetic assays

Communities were sampled in triplicate by metal cores (3.8 cm^2 surface area) and incubated at the site inside Whirl-pak® bags (60 ml. *Nasco*). In order to maintain conditions as natural as possible10 ml of pond water was introduced in the bag and the tracer ($Na_2\ ^{13}CO_3$) was added at an approximate concentration of 10% of the natural concentration of the light isotope.

Incubations were carried out in situ. For obtaining different irradiance conditions neutral filters were used. Neutral filters had a result of 100, 63, 24, 15, 7.8, 4.5, 1.5, 0.8, 0.5 and 0% of incident light transmitted during incubations.

Photosynthetic activity was measured as $H^{13}CO_3^-$ (98% ^{13}C. Isotec) uptake, using ^{13}C as a tracer (Ariosa et al. 2006). Two-hour incubations in presence of ^{13}C were stopped adding 3 ml of HCl 1N per bag to promote the non assimilated ^{13}C transformation to $^{13}CO_2$ and to volatilize to atmosphere. Acidified samples were rinsed with GFF-filtered pond

water ad dried. Afterwards, they were stored frozen and shipped to laboratory. Carbon uptake followed the same protocol described above, but without light dimming.

In laboratory, samples were weighted and completely dried out at 60ºC during 24 hours. After grinding, isotopic enrichment was determined by IRMS Micromass-Isochrom mass spectrometer.

Nitrogenase activity

Nitrogenase activity was determined by the Acetylene Reduction Assay (ARA) as described by (Fernández-Valiente et al. 2001). In brief, two cores of 22 mm in diameter of each community, supplied with 100 ml of GF/F filtered surrounding water were added to three 250 ml flat tissue culture flasks (IWAKI, Asahi Glass Co., Ltd.) stopped with rubber stoppers. To each flask, acetylene was added at 10% (v/v) concentration. After four hours, ethylene produced by the nitrogenase from acetylene reduction was collected by extracting gas from the incubation flasks and stored in 10 ml vacuum tubes (Vacutainer). The tubes were stored at 4ºC and film wrapped for shipping to the laboratory. There, ethylene concentration was determined by a gas-chromatograph (Shimadzu GC-8A) equipped with a flame ionization detector using a Porapak N80/100 column at the laboratory. These assays were only carried out in Antarctica, because acetylene transportation was limited in the Arctic.

Statistical and numerical analysis

Photosynthesis vs. irradiance curves were fitted to the Platt photosynthesis model (Platt et al. 1980) which follows a hyperbolic tangent equation, using Sigmaplot software (Systat Software Inc.). Here, Ps is defined as the estimated maximum photosynthetic rate, α as the estimated initial slope of the curve (a measure of photosystems efficiency) and Ek as the irradiance of saturation values (defined as Ps/α). Correlation original data and the modelled ones were expressed using the Pearson correlation coefficient (r2). To

standardize irradiance values trough the sampling sites, this is expressed as percentage of total incident irradiance.

Two approaches were made to assess the influence of environmental variables on the taxonomical composition of primary producers of the communities, one of them is a bipolar comparison and the other compiles more environmental variables only within the Arctic communities. In the comparison between both polar regions only conductivity, water temperature, pH, DIC, C/N, POC, PON, proportion of C and N by dry weight and natural abundance of 13C and 15N were considered. Therefore, an eco-climatic variable has been analyzed in parallel that is the number of days per year with temperature above 0 ºC at each site where ponds are located. Some of them (POC, PON, conductivity and proportion of C and N by dry weight) had skewed distributions and were log transformed. The aim of elected variables was in two ways, to estimate the pond conditions (exogenous environmental variables) and relate them with the microbial communities' C and N status (endogenous environmental variables). Those environmental variables were used to calculate a dissimilarity matrix between communities by standardized Euclidean algorithm. On the other hand, the binary matrix of primary producers´ presence on the communities was used to calculate the dissimilarity matrix between the communities composition via Bray-Curtis distance measures. Both dissimilarity matrixes were related by Spearman rank correlation (ρ) to assess the influence of environmental variables on the taxonomical composition.

A principal component analysis (PCA) was performed to display the environmental variables that explain the variance of the data and to select the environmental variables that will be included in the correlation analysis and the scores of the analysis plotted in 2D diagram which is an step forward of BIO-ENV analysis proposed by (Clarke and Warwick 2001) to relate the environmental variables with biotic data. Also, metric

multidimensional scales (MDS) plot was used to visualize the closest correlation with the biotic data. All of the procedures were carried out in SPSS v.17.

The method of choice for multivariate representation of community structure is often non-metric multi-dimensional scaling (MDS). This has great flexibility in accommodating biologically relevant (i.e. non correlation-based) definitions of similarity in species composition of many samples, and in preserving the rank-order relations amongst those similarities in the placing of samples in an ordination. Correlation-based techniques (such as Canonical Correlation) are then inappropriate in linking the observed biotic structure to measured environmental variables; a more natural approach is simply to compare separate sample ordinations from biotic and abiotic variables and choose that subset of environmental variables which provides a good match between the 2 configurations. In fact, the fundamental constructs here are not the ordination plots but the (rank) similarity matrices which underlie them: a suitable measure of agreement between 2 such matrices is therefore proposed and used to define an optimal subset of environmental variables which “best explains' the biotic structure (Clarke and Ainsworth 1993).

Consequently, this protocol was followed only with the Arctic communities due to the wider environmental data available. But the following variables were excluded from analysis due to the absence of some community data: SRP and NO3-. If appropriate, an ANOVA with the Tukey post-hoc test were used with a significance value of 0.05.

RESULTS:

Environmental variables

Physiochemical variables varied along wide ranges. Conductivity ranged from 421 to 50 µS cm-1 in Antarctic ponds and from 271 to 41 µS cm^{-1} in the Arctic ones, while pH ranged from almost neutral to alkaline (6.6-9.0) and again there is a remarkable difference between both polar regions (table 1). This pattern is not followed by DIC values, while the Arctic values ranged from 5.5 to 33.6 mg C l-1, the Antarctic ponds presented higher values, ranging from 16.8 to 82.8 mg C l-1indicating a quite different water chemistry and lithology. The Arctic DOC values ranged from 0.7 to 5.3 mg C l-1(table 1) but have not been analyzed in Antarctic samples. Those variables are considered hereafter as exogenous in the analyses, because they come from limnological data of the overflooding water. In the other hand, the endogenous variables hereafter are considered those exposed on table 2 as POC, PON, C/dW, N/dW, NA C, NA N, and C/N, due to only those variables were measured at each sampling site. Those values varied greatly between polar regions. Also, the variability in ponds within the same site does not present any pattern but are comprised on the same orders of magnitude across each microbial mat. These suggest the parallelism between described communities at both polar regions. Other measured variables with no differences between at both regions were PON (ANOVA; p-value=0.336), and NA N (ANOVA; p-value=0.496). On the other hand, POC values were different (ANOVA; p-value=0.016) and almost twofold on Arctic mats than the Antarctic communities, ranged from 57.75 to 226.67 and 16.49 to 94.56 gC cm-2, respectively. And also NA C values were statistical different (ANOVA; p-value=0.010). In this way, C/dW and N/dW presented significant differences in the bi-polar comparison (ANOVA; p-value=0.016 and 0.003, in that order). The two latter due to the different amount of sediments, stones and pebbles intertwined within biomass.

Table 1. Background of limnological data for the Arctic and Antarctic water bodies. Dissolved inorganic and organic carbon (DIC and DOC) values are from single measurements. * Not analyzed.

Polar region	Site	Coordinates (Datum WGS 84)	Pond	Water Temperature (°C)	pH	Conductivity ($\mu S\ cm^{-1}$)	DOC ($mg\ l^{-1}$)	DIC ($mg\ l^{-1}$)
Arctic	Ellesmere Island	82° 59.096′N-75° 24.194′W	W1	4.3	7.4	88.4	4.3	17.1
	Ward Hunt Island	83° 05.535′N-74° 08.263′W	W2	2.2	8.3	156.2	0.8	22.4
	Ellesmere Island	83° 02.642′N-75° 42.069′W	W3	12.3	9.0	179.4	5.3	19.5
	Ellesmere Island	83° 00.138′N-75° 02.342′W	W4	4.5	8.5	41.4	2.0	5.5
	Ellesmere Island	83° 00.433′N-77° 19.361′W	W5	10.2	8.5	86.6	0.8	9.0
	Ward Hunt Island	83° 11.000′N-74° 11.000′W	W6	1.9	7.3	114.0	0.7	14.4
	Resulote Bay (Cornwallis Is.)	74° 41.713′N-94° 59.334′W	R1	5.0	8.5	223.0	1.5	23.3
	Resulote Bay (Cornwallis Is.)	74° 43.048′N-95° 00.635′W	R2	9.1	8.5	271.0	1.8	33.6
Antarctica	Mars Oasis (Alexander Is.)	71° 52.695′S-68° 15.062′W	M1	13.0	6.6	50.0	*	24.0
	Mars Oasis (Alexander Is.)	71° 52.745′S-68° 15.118′W	M2	7.1	6.6	54.0	*	21.6
	Ares oasis (Alexander Is.)	71° 52.899′S-68° 15.435′W	A1	2.0	6.6	421.0	*	82.8
	Ares oasis (Alexander Is.)	71° 50.460′S-68° 13.550′W	A2	0.5	6.6	74.0	*	16.8
	Ares oasis (Alexander Is.)	71° 50.458′S-68° 13.486′W	A3	4.0	6.7	206.0	*	25.2
	Byers Peninsula (Livingston Is.)	62° 37,102′S-61° 42.234′W	SW	8.3	8.6	223	*	5.42

Table 2. Nutrient concentration in the water and Redfield ratios from the microbial mats assayed. SRP: Soluble reactive phosphorous. POC: Particulate organic carbon. PON: Particulate organic nitrogen. dW: Dry weight. NA: Natural abundance.* Missed value. ** Not analyzed.

Variable	SRP	Total P	Total N	$N\text{-}NO_3^-$	POC	PON	C/dW	N/dW	NA N	NA C	C/N by	N/P
Site	($\mu gP\ l^{-1}$)	($\mu gP\ l^{-1}$)	($\mu gN\ l^{-1}$)	($mgN\ l^{-1}$)	($gN\ cm^{-2}$)	($gN\ cm^{-2}$)	($mgC\ (gdW^{-1})$)	($mgN\ (gdW^{-1})$)	($\delta^{15}N$)	($\delta^{13}C$)	weight	by weight
W1	0.83	7.48	0.18	0.02	107	5.81	186.95	10.15	0.08	-18.95	18.42	24.59
W2	*	4.53	0.15	*	69.83	4.3	160.6	9.9	1.17	-25.32	16.22	32.65
W3	0.67	10.73	0.33	0.004	105.56	4.81	184.45	8.4	0.54	-25.03	21.96	30.3
W4	0.99	5.47	0.06	*	226.67	1.45	390.15	24.95	-1.3	-23.42	15.64	10.43
W5	0.51	4.38	0.16	0.05	57.75	3.57	116.45	7.2	1.52	-18.09	16.17	35.41
W6	0.67	3.13	0.09	0.04	199.58	8.51	172.35	7.35	0.05	-22.31	23.45	29.71
R1	*	5	0.13	*	154.01	9.14	213.9	12.7	1.47	-21.99	16.84	26.2
R2	0.35	1.88	0.2	0.05	212.27	8.84	168.15	7	0.81	-25.84	24.02	105.32
M1	**	**	**	**	94.29	7.26	552	42.5	3.2	-19.51	12.99	**
M2	**	**	**	**	94.56	5.58	499	29.5	-0.62	-11.11	16.93	**
A1	**	**	**	**	66.02	4.17	261.5	16.5	2.81	-11.71	15.85	**
A2	**	**	**	**	43.96	4.69	225	24	-0.18	-19.02	9.38	**
A3	**	**	**	**	16.49	1.19	625	45	-1.16	-22.82	13.89	**
SW	**	**	**	**	45.49	3.8	228.4	18.3	2.5	-13.8	12	**

All the physiochemical and biotic measures were performed and analysed by statistical tools in order to relate the environmental variables, which explain most of the variance of the data, with biotic data. Matrix dissimilarities correlated by Spearman rank correlation (ρ) between all pond environmental variables and biotic data of both polar regions were significant at 0.01 level but only explained 51% of the variance (ρ=0.51; p-value<0.01). The correlation did not improve when only endogenous environmental variables are displayed (ρ=0.51; p-value<0.01) and present a poor correlation when only exogenous variables are taken (ρ=0.36; p-value=0.01), that means the bulk amount of information (environmental variables) results in flawed accuracy of the analysis, so only a subset of variables are needed to improve it. This result is confirmed by the PCA that marks four components to explain 80.2% of the total variance. The component matrix of PCA was plotted in two dimensions to choose the subset of variables (figure 4) that explain the dispersion of the data. Conductivity, C/N, C by dry weight and latitude were selected and the Spearman rank correlation coefficient lightly improves (ρ=0.54; p-value<0.01). Attending to those variables, the prediction capacity (Spearman correlation) is very low, only in the 54% of the variability. Then a new variable was added, that is the number of days with temperature above 0 °C. Here, the spearman correlation improves to ρ=0.61 (p-value<0.01). So, the best match between physiochemical variables and biotic data is explained by a combination of endogenous variables (C/N, POC, PON c/dW, NdW, NA C and NA N) with site characteristics, those are latitude and number of days degrees above 0 °C (figure 7) that groups the ponds by site location characteristics (figure 4c). This new approach has a Spearman correlation similar (ρ=0.59; p-value<0.01) and point it out as the best physiochemical variable combination, although the prediction capacity is still low.

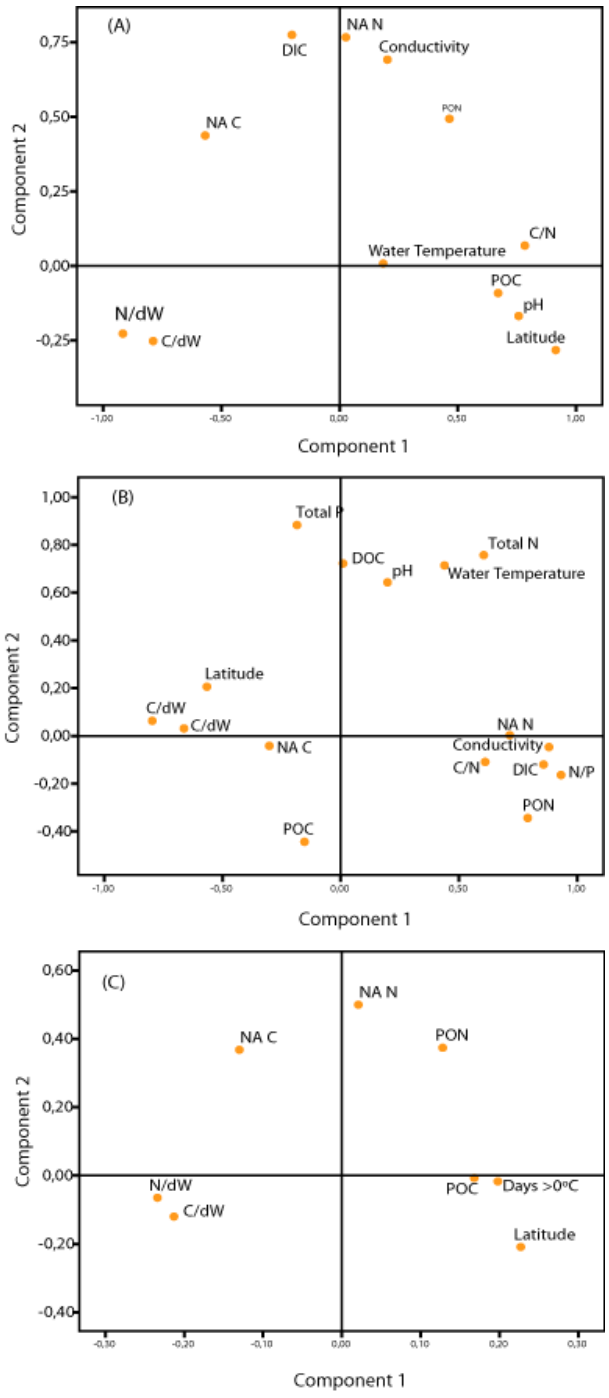

Figure 4. Principal component analysis (PCA) plots of the physiochemical variables comparing each of the 14 ponds assayed from both polar regions (A) and only from Arctic ponds (B). (C) PCA of combination of endogenous variables and macroscale variables (latitude and number of days above 0 °C per year).

Same procedure was carried out only with Arctic ponds. Plotted data of the two main components of PCA displays a similar distribution of variables, but some of them changed their influence (figure 4b) and the Spearman rank correlation between exogenous environmental variables are not significant (ρ=-0.265; p-value=0.173) and neither with the endogenous ones (ρ=-0.012; p-value=0.950). Moreover, when a subset of variables is selected (e. g. total P, N by dry weight, N/P and POC), again the analysis is not significant (ρ=0.272; p-value=0.16).

Instead of normalized data, the combination of physiochemical variables change the influence of some of the variables. The most clear is water temperature that has no influence in the bipolar comparison but increases its influence only at Arctic ponds survey (figure 4a and 2b).

Community composition

Total species richness in individual ponds ranged from 5 (A2) to 10 (SW) and from 5 (W4) to 18 (W6 and R2) at Antarctica and Arctic respectively, it is clear the large difference between both polar regions (ANOVA, p-value=0.002) (table 3). In this study Resolute Bay (Cornwallis Is. about 74 °N) showed the richest diversity in primary producers within the microbial mats and Alexander Island (about 71 °S) showed the lowest primary producer diversity in the study. The microbial mats from all ponds were dominated by filamentous cyanobacteria of the order Oscillatoriales, but W4 was mainly composed by Nostocales. Moreover, the presence of green algae is preferably on microbial mats from the Arctic, thus the presence of cyanobacterial groups cope the most of the biomass on those assemblages. Also in SW, the microbial mat from Byers Peninsula, the green algae had great importance on the food web and the diversity of cyanobacteria was larger than the mats described from Alexander Island. Composition of the communities varied greatly between Arctic and Antarctic ponds, drawing attention to SW (sited at Byers Peninsula) and W4 (sited at Ellesmere Island). Both microbial mats presented the largest variability of Antarctic site assayed and the lower on Arctic sites, respectively. This result was as well displayed on the MDS plotted data with a stress value of 0.17 (Kruskal´s stress formula 1) (figure 8a) where SW and W4 look as if they were outliers between both polar groups. Those presented the highest diversity in Antarctica (SW) and the lowest on the Arctic assemblages (W4). The Bray-Curtis standardized dis-similarities ranged from 0.11 between A2 and A3 that means those share the most of the organisms and 0.91 between R1 and W4, which means those mat are absolutely different and only share a few species (table 1).

Table 3. Genera-Species composition of green algae and cyanobacteria assemblages in each of the 14 ponds.

Order	Genera/species	Pond													
		W1	W2	W3	W4	W5	W6	R1	R2	M1	M2	A1	A2	A3	SW
CHROOCOCALES	*Aphanocapsa* sp.	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	Aphanothece sp.	+	+	-	-	-	+	+	+	-	-	-	+	-	-
	Chlorogloea sp.	+	-	+	-	+	+	-	-	-	-	-	-	-	-
	Chroococcidiopsis sp.	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	Chroococcus sp.	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	Gloeocapsa sp.	-	-	+	-	-	+	+	+	-	+	-	-	-	-
NOSTOCALES	*Dichotrix* sp.	+	-	-	-	-	+	+	+	-	-	-	-	-	-
	Nostoc spp.	+	+	+	+	+	+	-	+	+	-	-	+	+	+
	Tolypotrix sp.	+	-	-	-	-	+	+	+	+	-	-	-	-	+
OSCILLATORIALES	*Crinalium* sp.	+	-	-	-	-	-	+	-	-	-	-	-	-	-
	Homeotrix sp.	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	Leptolyngbya spp.	+	+	+	+	+	+	-	+	+	+	+	+	+	+
	Lyngbya sp.	-	-	-	-	+	-	+	-	-	-	-	-	-	-
	Microcoleus sp.	-	+	-	-	+	-	-	+	+	+	+	-	-	-
	Oscillatoria sancta Kützing ex Gormont	-	-	-	-	-	+	-	+	-	-	-	-	-	-
	Phormidium autumnale (Agardh) Trevisan ex Gomont	-	+	-	-	+	+	+	+	-	-	-	-	-	+
	Phormidium koettlizi Fristch	+	+	-	-	+	+	+	+	-	-	-	-	-	-
	Phormidium murrayi (W. et G.S. West) Anagnostidis et Komarek	-	+	+	-	-	-	-	+	-	-	-	-	-	+
	Phormidium pseudoprestleyi Anagnostidis et Komarek	+	-	-	-	-	+	-	-	-	+	+	+	+	+
	Phormidium subproboscidea W. et G.S. West	-	-	-	-	-	+	+	-	-	-	-	-	-	+
	Scytonema sp.	+	+	-	-	+	+	-	+	+	+	+	+	+	-
VOLVOCALES	*Chlamydomonas* spp.	-	+	+	+	+	-	-	+	-	+	+	-	-	+
	Chloromonas spp.	+	+	+	-	-	-	+	-	+	-	+	-	-	+
ZYGNEMATALES	*Closterium* sp.	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	Cosmarium sp.	+	+	+	-	-	+	+	+	-	-	-	-	-	-
	Mesotaenium sp.	+	+	+	-	+	-	+	+	-	-	-	-	-	-
	Mougeotia sp.	-	-	+	-	-	+	-	-	-	-	-	-	-	-
	Zygnema sp.	+	-	+	-	-	+	-	+	-	-	-	-	-	-
KLEBSORMIDIALES	*Klebsormidium* spp.	-	-	-	-	-	-	+	-	-	+	-	-	-	+
TOTAL		16	14	13	5	11	18	16	18	6	7	6	5	4	10

Physiological assays

Nitrogenase activity was only assayed at the Antarctic ponds; those presented low activity, near the detection limits. M1 pond (Worland pond) presented a remarkable activity (7.6±0.8 μmol ethylene m^{-2} h^{-1}), and SW pond showed slightly higher N_2 fixation rates than those of Alexander Island (10.9±0.9 μmol ethylene m^{-2} h^{-1}) (figure 5).

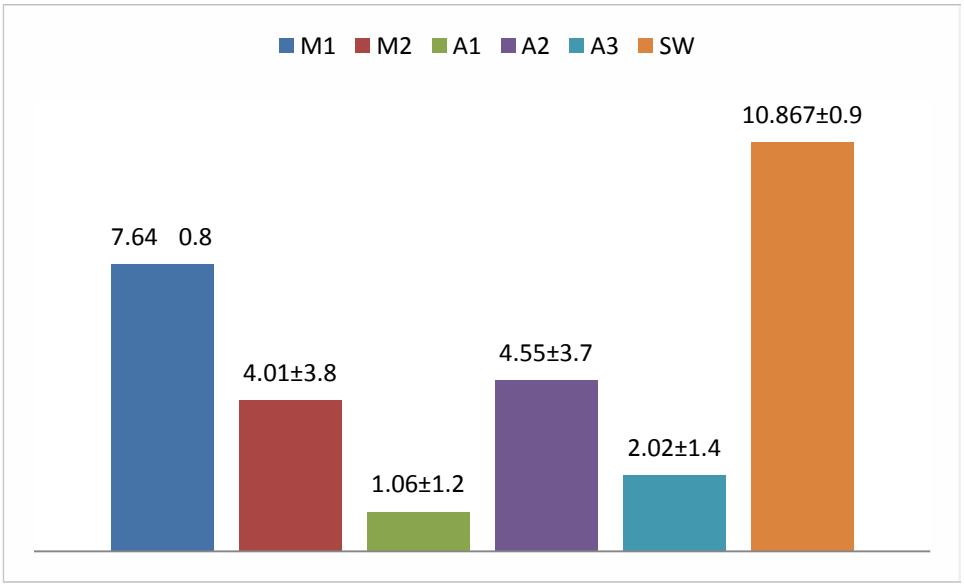

Figure 5. Acetylene reduction assays of ponds from Mars (M1 and M2) and Ares Oasis (A1, A2, A3) (Alexander Island, Antarctica) and a microbial mat from Byers Peninsula (SW)all expressed as μmol Ethylene $m^{-2}h^{-1}$.

The PvI curves (figure 6) did not present any clear pattern between communities growing on ponds at different latitudes. Leaving out the PvsI curve of SW, which showed similar behaviour but higher Ps and α values than the communities form Alexander Island (table 4); Ps values of the curves showed only slight differences between communities. W1, sited in Mars Oasis (72 °S) showed the smallest value (figure 6). Thereafter, between R1, A1 and W6 there were not significant differences. α values marked the same situation without any remarkable difference between ponds assayed with the exception of SW, and W1 which α value is one order of magnitude lower. E_k values mirror this situation (figure 6) and show the percentage of irradiances transmitted at which the communities reached photosynthetic saturation levels (table 4). Again, SW pond is presented as an outlier

comparing mats at different latitudes. In this way, Photosynthetic and N_2 fixation assays face up the variability of community dynamics between communities sited nearby.

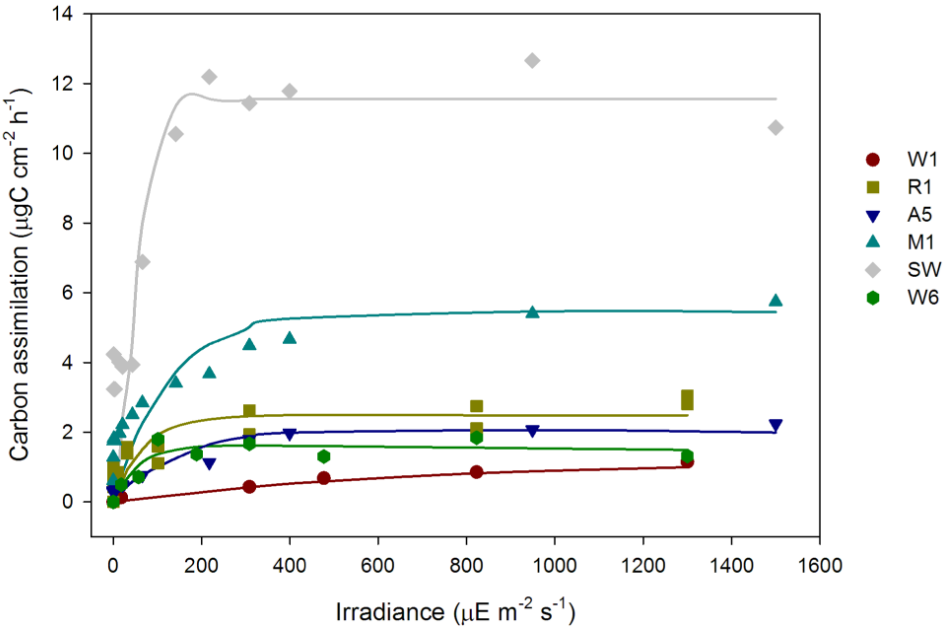

Figure 6. Photosynthesis vs. temperature curves fitted to Platt model (Platt et al. 1980) from the microbial mats assayed in Arctic and Antarctica. Dotes are the average of three samples and irradiances are standardized for both polar regions.

Parameters	*Microbial mats-Ponds*					
	M1	**A1**	**W1**	**W6**	**R1**	**SW**
P_s(µg C cm^{-2} h^{-1})	5.5	2.1	1.1	1.6	2.5	11.56
α (µg C cm^{-2}h^{-1} (µEm^{-2} s^{-1})$^{-1}$)	0.7	0.2	0.02	0.4	0.5	1.41
E_k (µEm^{-2} s^{-1})	124.5	142.5	648.7	55.9	67.6	123.3
Pearson´s data correlation (r^2)	0.96	0.97	0.99	0.91	0.90	0.96

Table 4. Estimated photosynthetic parameters from 5 communities. Maximum photosynthesis (P_s) values are in µg C cm^{-2}h^{-1}. Photosynthetic efficiency (α) units are (µg C cm^{-2}h^{-1} (µEm^{-2} s^{-1})$^{-1}$). These values were fitted to Platt et al. (1980) model for each photosynthesis versus irradiance curve. E_k marks the irradiance of saturation. Pearson correlation value (r) is identified by the fitted boundary of the models.

DISCUSSION:

Taxonomical composition differences between microbial mats from both polar regions show a major predominance of green algae on Arctic mats, which is not exhibited by the Antarctic communities (table 3), probably due to the mid-summer particular conditions when sampling were carried out. Polar environmental cold conditions promote the growing of organisms with psychrophilic profiles, as described profusely about green algae (Morgan et al. 1998; Hoham et al. 2008; Hoham et al. 1993; Loppes et al. 1996; Ling and Seppelt 1998, 1993). Also this difference could be prompted by the influence of higher trophic levels with different diets and predation rates or only by ecological succession. While in the Antarctic mats metazoans were scarcely represented in some mats, some of the lakes or ponds where Arctic mats grew, were under the influence of many metazoan groups including in some cases fishes (e. g. Meretta Lake (R1), and PCSP pond (R2)) (Rautio et al. 2008; Maslen and Convey 2006; Rautio and Vincent 2006). Nevertheless our results clearly indicate that the mat communities' composition is quite different in the polar regions we have investigated. This bi-polar composition pattern is also displayed in the multidimensional scaling plot proposed (figure 4a).

Contrastingly, the physiological activities that we measured (photosynthetic parameters and N_2 fixation) apparently did not follow this diversity pattern. The photosynthetic parameters measured in this study are in the same range than those published for other microbial mats in polar regions (Vincent 2000b). However, the N_2 fixation values of Alexander Is. spots and Byers Peninsula, are low in comparison to previous assays on Byers Peninsula (Velázquez et al. 2011), but are on range of N_2 fixation assays from McMurdo Ice Shelf microbial mats (Fernández-Valiente et al. 2001). N_2 fixing capacity is widespread within cyanobacteria although the genera in the order Nostocales, are the most ubiquitous organisms able to carry out an ecological significant activity in benthic communities widespread across polar regions (Paerl et al. 2000). So, in spite of the low rates found, N_2 fixation could be the main N input in the ecosystem.

Special attention might be paid to similarities of physiological results at every latitude instead of the great differences in the communities´ composition. This fact could be due to the physiological assays measured short-term activities of the microbial mats, which might not be a representation of the long term dynamics of each mat. Moreover, it addresses a scale divergence problem. The apparent physiological status (short term activities) measured cannot be assumed as a representation of the long term dynamics, since crucial environmental variations driven by the ice conditions can modulate the responsiveness of the organisms.

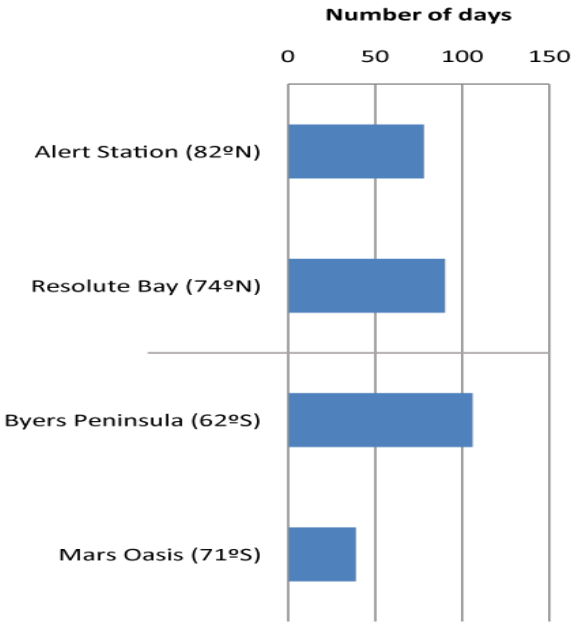

Figure 7. Number of days above 0 °C per year at every studied location. Alert Station is located 82°31'04"N-62°16'50" W in the North-west coast of Ellesmere Island. The Antarctic data come from 8 years series, but the Arctic ones come from 30 years series.

The climatic data show that there is a gradient in some relevant variables through the latitudes in the Arctic and Antarctica (figure 7). Surprisingly, this pattern is not followed by the microbial benthic communities nor in community composition neither in the single measure physiochemical conditions (Sutherland 2009). In the bipolar analysis, there is not a single key environmental variable responsible for the biological distribution, but a combination of a few of them is needed in the proposed multivariate analysis to identify the variation (figure 8). Thus, low correlations between environmental variables and biotic data point out the need of broader time scales proxies. Those proxies may properly explain the environmental background of the study sites, summing up those

variables that lead a given assemblage settlement. Nevertheless, the MDS graphic approach suggests that some of the physiochemical data match well with the biotic composition in large scale studies, that is the bipolar analysis of the present work (figure 4a), but are not fine enough to explain the variance between ponds sited at similar latitudes due to their internal variability (Stibal et al. 2007). In this way, a combination of microscale variable intrinsic to each mats studied combined with macroscale variables might explain better the community assemblage's differences (figure 8e).

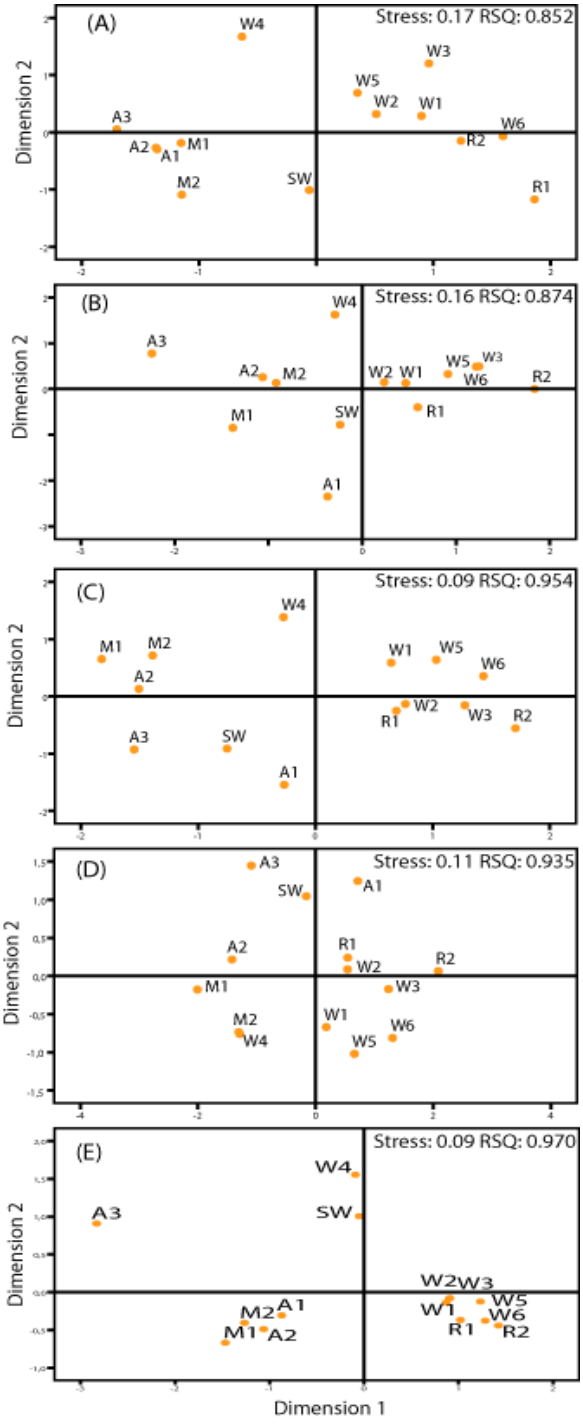

Figure 8. Metric multidimensional scaling (MDS) plots of primary producers of the communities' data from each of the ponds assayed. (A) MDS plot of cyanobacteria and green algae community data from each of the 14 ponds. (B) MDS plot of all environmental variables assayed from each pond. (C) MDS plot of a subset of abiotic variables (conductivity, C/N, C/dW, and latitude). (D) MDS plot of a subset of abiotic variables (conductivity, C/N and C/dW). (E) MDS plot of a subset of abiotic variables (conductivity, C/N, C/dW and latitude) combined with a macroscale climatic variable (number of days above 0 ℃ per year). A stress value of 0.09 indicates a very good representation of the 14 ponds in two dimensions.

Usually it is considered that biodiversity and latitude are inversely related, in the way that ecosystems located at lower latitude show larger diversity and vice-versa. This pattern is attributed to a gradient of temperature, humidity and duration of the summer season (Smith 1994; Kappen 2004; Convey 2001). In fact, the highest rates of terrestrial biodiversity around the Antarctic Continent are given in the Antarctic and sub-Antarctic Islands located on the periphery of the Continent (Convey and Stevens 2007) at lower latitudes. Only in certain "oasis " of life in the Antarctic Continent, usually related to liquid water availability and its consequences (Priscu et al. 1998), converge conditions for a few organisms to settle forming communities with some self-regulatory capacity that allow the establishment and development, softening the effect of the dominant physical factors (Camacho 2006). So, in Maritime Antarctica the patchy distribution of microbial communities is related to proper soil conditions and liquid water availability (Maslen and Convey 2006). On polar regions, these conditions converge on ecosystems as lakes and ponds where physical constrains are not so harsh. Thereafter, the patchy geographical distribution of those sites is not subjected to any latitudinal gradient. Here, the variable “Latitude”, on the analysis, works as a grouping parameter that sum up the possible biogeochemical background on the different sites, as well the variable “number of days >0 °C”. The latter variable points out the presence of a climatic gradient to the poles, but this pattern is not followed by the cyanobacterial and green algae communities’ composition and by physiochemical variables within polar regions (figure 4b), those have different time scales and are intimately linked (Stibal et al. 2007). On the other side, Shuterland (2009) pointed out the variance of physiochemical variables across different time spans, by themselves, are unable to predict any community composition and correlations usually are unsuccessful. Again, a scale disparity issue, that is trying to predict microscales consequences from macroscale variables, faces up that a combination of a few variables of different time and influence scales provides more accurate analysis. So, it addresses the importance of selection proper proxies from different approaches.

In conclusion, every community compiles a single background of eco-climatic variables e. g. number of days with maximum temperatures above 0 °C or balance of degrees above 0 °C per year that regional conditions exert. This is one of the underlying ideas from the biome's theory (Odum 1945). Also, the biogeochemical account of every area provides the convergence of proper conditions for life flourishing (Chown and Convey 2007). This fact seems to be very relevant in polar regions where the life supporting factors may remain close to their boundaries, and slight differences can push out some organisms (Wall 2007). Nowadays, the development of mathematical and statistical (e.g. multivariate analysis proposed here) tools does help working out the relationships underlying beneath every single benthic assemblages from polar regions. As complementary variables, it is also important to bear in mind the ecological relationships background between organisms (processes as colonization or new species invasions) and their single biological capabilities, in some cases derived from strain differences. So, those constraints might be taken into account for further analysis with the aim to relate environmental condition with biotic data.

Acknowledgements: This work has been possible due to logistic support of *British Antarctic Survey* on Alexander Island (Antarctica) and the Warwick Vincent Team at *Université Laval* that hosted me and provided the facilities to reach High Canadian Arctic sites. Also the *Unidad de Tecnología Marina (UTM-CSIC)* help us in transportations within Antarctica.

Capítulo 2/Chapter 2

Temperature influence on microbial freshwater assemblages at Maritime Antarctica.

"El curioso mundo en el que vivimos es más maravilloso que conveniente, más hermoso que útil, más digno de ser admirado que disfrutado y usado"

The commercial spirit of modern times. Henry D. Thoreau, 1837

ABSTRACT

One of the prerequisites for life in seasonally frozen environment is the ability to maintain viability until the next period of thaw and at that point being ready for growing, resuming photosynthesis and other physiological processes within minutes to hours after thawing. The present study shows the very beginning effects that temperature exerts on a microbial mat mostly composed by Cyanobacteria and sited at Maritime Antarctica (Lagoon Island, Antarctic Peninsula). The temperature regime at the beginning of every growing season (summer) may exert shifts in benthic community´s composition of waterbodies across Maritime Antarctica. In the present study, our aim was to compare how the temperature leads community composition at the beginning of the Antarctic season in the Maritime Antarctic and how the ecological succession and communities assemblages are influenced by temperature. This was made in environmental controlled chambers in parallel with field sampling measures. We hypothesize that temperature regime is a key factor in promoting microbial mats matrix every year, when environmental conditions become warmer overcoming slight differences in terms of biomass accumulation and further biogeochemical interactions. Thus, particulate organic carbon (POC) measures, here used as a proxy of mat growing, illustrate the slow dynamic of those benthic communities. The results point out the importance of temperature regimes at the beginning of every summer season in order to get new stages on the ecological succession of the communities.

INTRODUCTION

Extreme cold has shaped the Antarctic environment and the microbial communities that live within it. Even throughout summer, air temperatures at the margins of the Continent and in the Maritime Zone lie between -10 and +5 °C (Vincent 2000b). The dramatic and discontinuous change in the physical properties of water over this range, in particular at its freezing point, has a far-reaching impact on the chemical and physical characteristics of all the potential habitats throughout the region. These environmental effects restrict the organisms that can inhabit there, and severely limit the timing and intensity of biological processes (Vincent 2000b). The definitions of psychrophiles and psychrotrophs follow those by Morita (1975) and Gounot (1986), mainly developed with reference to Bacteria: both psychrophilic and psychrotrophic organisms have the ability to grow at 0 °C. Thereafter, psychrophilic organisms have an optimum temperature for growth of c. 15 °C or lower, and a maximum temperature for growth of near 20 °C, whereas psychrotrophic organisms have an optimum temperature for growth above 20 °C.

Cold temperatures above freezing point may also exert decisive influence on the activity and species composition of Antarctic communities. At a cellular level these effects operate in part through the Arrhenius relationship between chemical reaction rate and temperature. Some of these responses to cold operate at the cellular or macromolecular level. The extent of hydrogen-bonding of proteins with water molecules is a determinant of their catalytic activity and this surrounding water structure is sensitive to temperature.

Low temperatures may have a differential effect on the biological components of the community. The effects of low temperature may strongly interact with other environmental variables, e.g. light intensity or day length. Those consequences may further generate no-linearity between temperature and the presence of certain taxa. They also influence the usefulness of traditional descriptors of microbial activity. Microbial mats of polar regions, with special emphasis in Antarctica, are good models to address the influence of physiochemical characteristics of the environment. The cyanobacterial-based

communities are assembled by the interactions between organisms and their trophic webs. Usually, those share the ecosystem structure, regularly setting up on a well developed matrix of thin cyanobacteria and a cohort of other autotrophic organisms which support short trophic webs. Chemical characteristics are influenced by the surrounded area and are fed back from the physical and biological organization of the community. Here abstractions of natural phenomena, like competition or ecological succession, can be approached by the diversity of scenarios developed from every single mat community background which show the emergence of self-organized and complex behaviours from the interactions of simple agents (Wynn-Williams 1996).

Nevertheless, other factors may strongly influence ecological succession. Stabilization of pristine soil habitats by various microbiota (Davey et al. 1991; Upton et al. 1990; Wynn-Williams 1993) and consequential modification of local microenvironmental condition (de los Rios et al. 2003) have been found important precursors encouraging further ecological developments by heterotrophic microbiota. However, a "classical" view of establishment and community development as a linear succession process may not be always appropriate. In certain situations, redistribution of "dead" organic matter into newly exposed and unoccupied habitats can encourage new colonization processes and maintain populations of higher trophic levels before the establishment of their expected primary food source. That is very common in colonization processes after glacial retrieving (Hughes et al. 2006). However, the stochastic arrival and development of key species with essential physiological and biochemical capabilities for establishment at a site may itself alter the environmental conditions and permit establishment of other species. The Maritime Antarctic Region experiences extended periods of freezing during winter, and rainfall plays an important role. Throughout the region water availability varies spatially and temporally, with large variations occurring from year to year. At a broad scale, levels of tolerance of different temperature ranges vary across the different

microbial mats and their biogeochemical background. So, long-term temperature records exert communities' composition and ecological dynamic (Wynn-Williams 1990).

In the present study, our aim is to compare how the temperature leads community composition at the beginning of the Antarctic summer season in the Maritime Antarctic influence zone and how the ecological succession and communities assemblages are influenced by temperature. This was made in environmental controlled chambers in parallel with field sampling measures. We hypothesize that temperature regime is a key factor in developing microbial mat matrixes every year, when environmental conditions become warmer overcoming slight differences in terms of biomass accumulation and further biological interactions.

MATERIALS AND METHODS

Study site

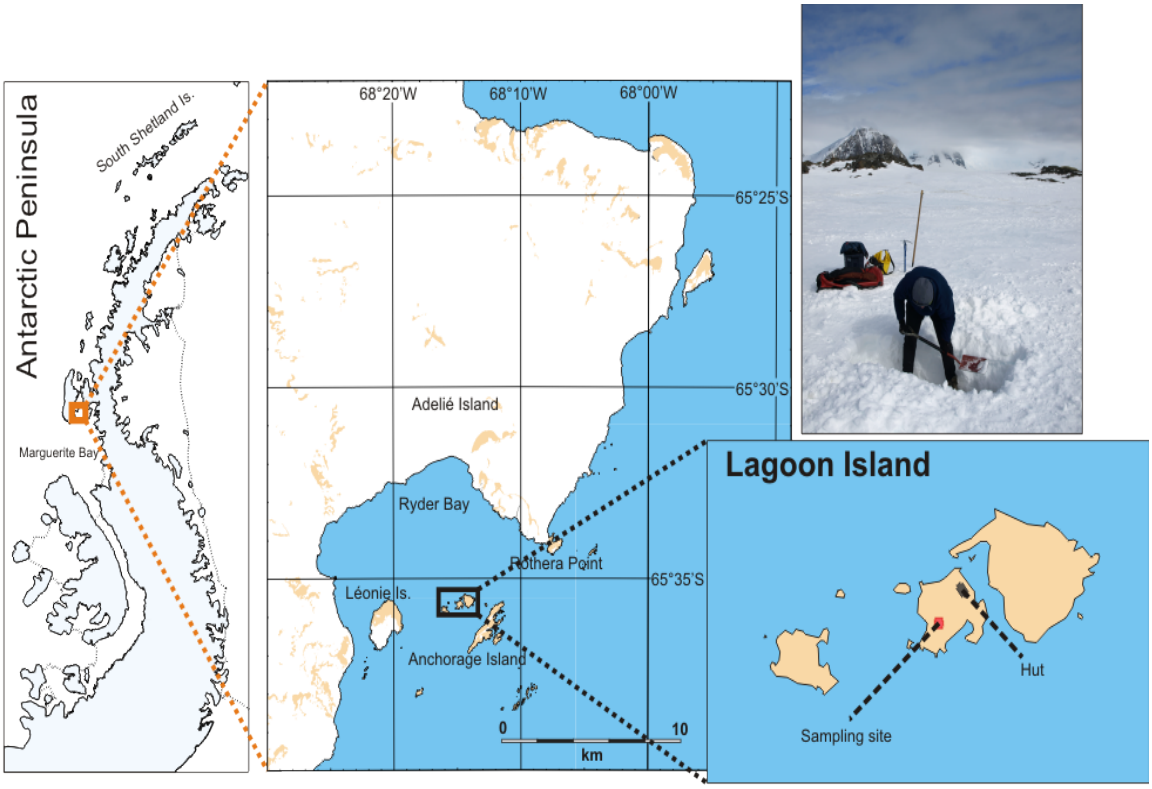

Figure 1. Map of Ryder Bay (northern Marguerite Bay), showing Rothera Point, Leonie, Anchorage and Lagoon Islands (67°35´S, 68°16´W). (B) works on the assayed benthic community of Lagoon Island.

Experiments were carried out from mid November of 2008 until mid-December of 2008 in a pond sited at Lagoon Island (67º 35.742´S- 68º 14.855´W) that is located at Ryder Bay (Northern Marguerite Bay), opposite to Rothera Research Station (figure 1). Lagoon Island (around 1 km^2) consists of Upper Jurassic volcanic rocks (quench-brecciated lava) (Dewar 1970). Much of the island, down to rocks just above high water, is covered by a dense, well-developed lichen fell-field. However, raised beach terraces on the island's eastern slopes are locally dominated by the vascular plants *Deschampsia antarctica* and the moss *Polytrichum alpinum*, whilst west-facing damp gullies and slopes are covered by a moss carpet dominated by *Drepanocladus uncinatus, Brachythecium austro-salebrosum* and *Andreaea* spp. Moist rock faces are festooned with large thalli of macro-lichens (notably

Umbilicaria spp. and *Usnea* spp.) (Convey and Smith 1997). Some other studies have been carried out on the Island; Furthermore, in McDaniel and Emslie (2002) were described some abandoned penguin colonies on the islands.

Sampling techniques and samples handling

Table 1. Conditions of the bulk snow bank over the surveyed community. Measurements were taken after thawing at room temperature. Snow temperatures were recorded at 5 cm depth.

Date	**15/11/2008**	**23/11/2008**
Snow temperature (ºC)	-0.7	-0.5
Specific conductivity (µS cm^{-1})	112	48
Conductivity (µS cm^{-1})	84	41
pH	3.5	5.16
O_2 Saturation (%)	90.5	93

A temperature growing survey of the assemblage capabilities was made from a microbial mat collected below of more than 1 m of snow and the aim was to compare how the temperature drives the benthic communities' composition. The first intention was to make a complete physiological profile and follow the evolution of the natural assemblage in parallel with incubations into climatic chambers. Unfortunately, logistical problems avoided this but the experiment was run using different growing proxies (described below). The benthic community was, at the first sampling moment, under a bulk snow bank about 70 cm depth. The community was included into an ice bottom layer about 15 cm depth. This snow and ice cap was transformed by temperature regime several times during the experiment and some layers were as well windblown, but the ice cap forthwith above the community still remained at the last sampling moment. Also, a strong smell of urea was persistent throughout all sampling collections.

After digging the benthic community out, samples were taken immediately to Rothera Station laboratories to initiate the experiments. Microbial community was gently homogenized and mixed with melted snow. Then, 10 ml of this seeding solution (table 1)

were filtered by glass fibre filters GF/F (Whatmann), using them as supporting matrix. So, a total number of 27 filters were prepared in petri dishes. Those aliquots were separated in groups of nine and incubated at three different temperature treatments within climatic chambers. Those at 1, 5 and 15 °C over 24 hours light cycles, to imitate the natural conditions. Also, thawed and GF/F filtered snow was used as culture media in order to keep all the nutrient ratios as similar to the natural ones as possible, irrigating them frequently to avoid desiccation and nutrient deficiency promoted by culturing the cores.

Weekly, 5 mm (inner diameter) cores were randomly collected from each temperature treatment for taxonomical monitoring and further physiochemical analysis. Field sampling events at Lagoon Island were carried out in parallel to compare the results with those from temperature treatments at the laboratory.

Particulate organic carbon (POC) and nitrogen (PON) values of every temperature treatment and field samples were measured from dried samples by using an Elemental Analyzer LECO CHNS-932.

Air temperature records within the vicinity of Rothera Research Station were summed as days with temperature above 0 °C from the last 8 years records. Those records were compared to other area within Maritime Antarctica, that is Byers Peninsula, which is sited about 700 km to the North and considered as one of the hot spot biodiversity of ice free areas in Maritime Antarctica (Rautio et al. 2008) where microbial mats are profusely represented (Fernández-Valiente et al. 2007). Physiochemical snow records were measured by a multiparametric probe (YSI 6920) after melting in order to have a broad image of the environmental conditions of the community. Unfortunately, only two records of melted snow were able to be taken due to logistics restrains.

Microscopic enumeration

For taxonomical description and enumeration, samples were counted at Rothera Research Station laboratory. For cyanobacterial taxonomic determinations the taxonomic keys of Anagnostidis and Komarek (1988, 1989) were used. Biological estimations were made at

every microscope field in two steps. First, low magnification (10x) was used to identify metazoan groups under UV fluorescence filter, then green algae abundance were assigned to four categories (sporadic, present, abundant and dominant). Second, under green fluorescence and higher magnification (20x), the cyanobacterial assemblages covertures on the filters were estimated by microscopic observation at every microscopic field while they were taxonomically described. These measurements are considered here as proxies of community development, because those assemblages are usually structured on a cyanobacterial matrix. So, the first stage for community growing is to set up a cyanobacterial matrix. To standardize the measures, three replicates of the same samples were examined by two different researchers independently. Those resulted on a less than 5% of difference in surface coverture. The cores for cyanobacterial coverture estimation were previously selected to carry out single-blind determinations by different researchers, to avoiding bias due to the analyst. At the same time, the field sampling protocol of biomass collection, homogenized with melting snow and filtered by glass fibre filter GF/F was followed to compare the development of the natural assemblage with temperature incubations at laboratory.

Data analysis

Where appropriate, paired t-test was used to compare replicated measurements. Where data did not conform to assumptions required for normality testing, Mann-Whitney Rank Sum Test or Kruskal-Wallis test were used. SigmaPlot 11.0 (SSPS, Inc.) was used for all statistical procedures and figure production.

RESULTS

Physiochemical conditions

The aim of the experiment was to show how natural assemblages of benthic communities from Maritime Antarctica start their growing season year after year. For this purpose the comparison between the development of natural community and the temperature treatments incubations (those seeded from the natural one) have shown the different dynamics exerted by short-term temperature conditions. The measurements of snow melting conditions define a very stable situation while community is still under its influence (Table 1).

The long-term records temperature profile from Rothera Research Station mark 74 days per climatic year with temperature records above 0° C per year. That means only the 20.3 % of the year (2.4 months per year). As a comparative profile, at Byers Peninsula the temperature records score 106 days with temperature above 0 °C per year (3.5 months per year) that is a 30% of the year with temperatures over 0 °C.

Community composition

At the first sampling event, phototrophic assemblage was composed by a cohort of diatoms, Cyanobacteria, Chlorophyte and some metazoan as tardigrades, nematodes, rotifers and ciliates (table 2). Most of the cyanobacterial morphotypes were assigned to Oscillatoriales order, according to Anagnostidis y Komarek (1988), but only one Nostoc-like morphotype was found. Microbial mat matrix consisted of a thin cyanobacteria identified as *Leptolyngbya* sp. Also, a cyanobacteria with the morphotype J (1-1.5 µm in diameter) was profusely found; sensu Broady and Kibblewhite (1991) compared to the Oscillatorian diversity in Ross Island and Southern Victoria Land in Continental Antarctica. Thicker cyanobacteria from morphotype B (3.6-5.4 µm in diameter) were also present within the mat. According to Anagnostidis y Komarek (1988) all these morphotypes could be assigned to different species of genus *Phormidium* (Broady and Kibblewhite 1991).

Besides, many filament of apparently *Microcoleus* sp. were found and some rounded cells of *Chroococcidiopsis* sp also. Eventually some filaments of an unidentified cyanobacterium with a sheath about 10 μm in diameter were observed; those probably belong to the family Phormidiaceae because of its thick, lamellated and coloured sheath. Diatoms were also present but at low density. Abundant microcolonies of *Nostoc* sp. (5-6 μm in diameter) also appeared. Intermixed with the matrix filaments there were also many filamentous green algae which belong to *Klebsormidium* genus. (Chlorophyte) and also some rounded cells about 6-9 μm were found, those cryobiontic chlorophytes were assumed to be *Chlamydomonas* sp. and *Chloromonas* sp. Then, changes in composition were not displayed by the community. Furthermore, the taxa found at every temperature treatment follow broadly this pattern without any remarkable differences (table 3).

Table 2. Relative abundances of different taxonomic groups of the field samples from Lagoon Island.

Taxonomic groups	15/11/2008	22/11/2008	27/11/2008	06/12/2008
Chroococcidiopsis sp.	++	++	+	++
Leptolyngbya sp.	+++	+++	+++	++
Microcoleus sp.	+	+	++	+
Nostoc spp.	+	+	+	+
Phormidium autumnale (Agardh) Trevisan ex Gomont	+	++	+	++
Phormidium murrayi (W. et G.S. West) Anagnostidis et Komarek	+	+	+	-
cf. *Phormidium* sp.	-	-	+	+
cf. *Prasiola* sp.	-	-	+	+
cryobiontic Chlorophyta	-	-	+	+
Klebsormidium sp.	+++	+++	+++	+++
Diatoms	+	+	+	+
Ciliates	-	-	-	+
Nematodes	-	+	-	-
Rotifers	-	-	-	+
Tardigrades	+	+	+	+

Table 3. Relative abundances of different taxonomic groups from three temperatures assayed

Time period	Taxonomic entities	1 °C	5 °C	15 °C
6th day	*Chroococcidiopsis* sp.	++	++	++
	Leptolyngbya sp.	+++	+++	+++
	Microcoleus sp.	+	+	+
	Nostoc sp.	+	-	-
	Phormidium autumnale (Agardh) Trevisan ex Gomont	++	++	+
	Phormidium murrayi (W. et G.S. West) Anagnostidis et Komarek	+	+	+
	cf. *Phormidium* sp.	-	+	-
	cf. *Prasiola* sp.	-	-	-
	cryobiontic Chlorophyta	+	+	++
	Klebsormidium sp.	+++	+++	+++
	Diatoms	-	-	-
	Ciliates	-	-	-
	Nematodes	-	-	-
	Rotifers	-	-	-
	Tardigrades	+	+	+
13th day	*Chroococcidiopsis* sp.	++	++	++
	Leptolyngbya sp.	+++	+++	+++
	Microcoleus sp.	+	+	-
	Nostoc sp.	+	-	-
	Phormidium autumnale (Agardh) Trevisan ex Gomont	++	++	+
	Phormidium murrayi (W. et G.S. West) Anagnostidis et Komarek	+	-	+
	cf. *Phormidium* sp.	+	+	+
	cf. *Prasiola* sp.	-	-	-
	cryobiontic Chlorophyta	+	+	-
	Klebsormidium sp.	+++	-	-
	Diatoms	-	-	-
	Ciliates	-	-	-
	Nematodes	-	-	-
	Rotifers	-	-	-
	Tardigrades	++	+	++
27th day	*Chroococcidiopsis* sp.	++	++	++
	Leptolyngbya sp.	+++	++	+++
	Microcoleus sp.	+	-	+
	Nostoc sp.	+	-	+
	Phormidium autumnale (Agardh) Trevisan ex Gomont	++	++	+
	Phormidium murrayi (W. et G.S. West) Anagnostidis et Komarek	+	+	++
	cf. *Phormidium* sp.	+	-	-
	cf. *Prasiola* sp.	-	-	-
	cryobiontic Chlorophyta	+	+	-
	Klebsormidium sp.	+++	+++	+++
	Diatoms	-	-	-
	Ciliates	-	-	-
	Nematodes	-	+	-
	Rotifers	-	-	-
	Tardigrades	+	+	++

Population dynamic

Statistic analyses mark the similarity between coverture percentage of 1 and 5 °C treatments (Mann-Whitney. P=0.724) for field sampling-1 °C and field sampling-5 °C respectively), but results should be interpreted cautiously because the power of the performed test (0.050) is lower than expected. In contrast, there is statistically a difference between those and 15 °C incubation (t-test; P<0.001) (figure 2).

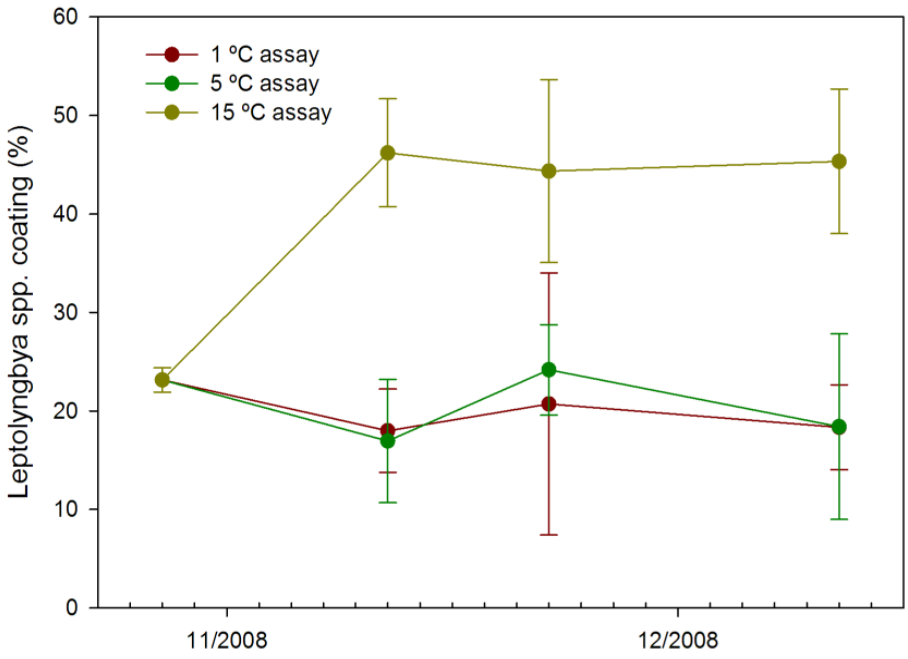

Figure 2. Matrix coating development comparing temperatures treatments (1, 5 and 15 °C). First sampling event gather the same initial point for all the treatments due to the natural assemblage was seeded the temperature incubations.

Due to the stability of the values of POC (26.1±0.29) and PON (6.5±0.002), the ratios of C/N have not undergone any change over all the samples (4.0±0.05) (table 4), indicating the stability throughout the experiment time span and as well that the system had no deficiency in N. This result was verified for both field samples and tests at different temperatures but probably address to P as the most limiting nutrient, which has not yet been measured on samples.

Table 4. Incubation conditions and C and N balances across the experiment performance (about 3 weeks). POC and PON are the averaged values across the experiment.

Treatment temperature (°C)	Treatment light ($\mu Em^{-2} s^{-1}$)	POC (%)	PON (%)	C/N By weight
1	50	26.13±0.14	6.55±0.001	3.99±0.02
5	72	26.17±0.18	6.55±0.002	4.00±0.03
15	46	26.28±0.32	6.55±0.002	4.01±0.05

Metazoans were a constituent feature within the community at every single temperature treatment and field samplings, but their random distribution along the samples is subjected to the experiment handling and there is no difference between temperature treatments (Post-hoc Kruskal-Wallis test P=1.00) attending to the hatched eggs and alive animals (table 2 and table 3).

DISCUSSION

Psychrotrophic characterization of cyanobacteria (Tang et al. 1997; Zakhia et al. 2008) and matrix development, in particular of *Leptolyngbya* spp. and *Phormidium* spp. has been described in many articles (Jungblut et al. 2010; Strunecky et al. 2010) all around the world with special emphasis in polar regions, however, its case is very important because it is the main component of the matrix of the Antarctic benthic communities and it takes relevance when the area is above freezing periods at some point through the year. The development of those communities is controlled by a complex array of external conditions, stress factors and interspecies influences. Microbial mats have a high turnover (Ellis-Evans and Walton 1990) and the success of individual species is difficult to predict, but the development of general patterns of community structure follows defined routes (Biggs 1996). Here, two different strategies are displayed. Cyanobacteria are further example. Some cyanobacterial taxa are usually perennial, capable of surviving desiccation and some are broadly resistant to freezing (e.g. *Phormidium*) (Velázquez et al. 2011). On the other hand, filamentous green algae (e.g. *Klebsormidium*) do not tolerate freezing or desiccation particularly well and for environment subject to freezing (fell-field soils and streams) only a tiny fraction of the cells survive the winter (Hawes 1989) these few cells, however, develop quickly once free water is again available (Velázquez et al. 2011), as is showed along all the microscopic surveys presented here.

There were no clear differences between temperature treatments in the C/N ratios, even though communities were beginning to differentiate themselves. The POC values, as a proxy of mat growing, as well illustrate little shifts on community short-term development perhaps it is showing a net balance about respiration and photosynthesis, therefore microscopic enumerations of *Leptolyngbya* spp. exhibit remarkable differences between temperature treatments, but those processes have not been measured so it might be interpreting cautiously. On the other hand, probably due to a nutrient deficiency and temperature regimes of the experiment, heterotrophic organisms, mostly grazers, were

not able to grow by nutrients deficiency on primary producers. This next stage on ecological succession, the so-called two-step chain situation within the Food Chain theory (HSS model) predicts very low-production systems containing only primary producers (Hairston et al. 1960). Those grazers are not active part of the community and should remain protected from the elements (low temperatures) as resistance forms. This points out the importance of temperature records in the Maritime Antarctic and in areas across the Continent, where the temperature range exceeds 0 °C on many occasions, these conditions serve as the basis for the development of entire communities every growing season. In fact, a decrease in chlorophytes viability was observed during the experiment at temperatures of 15 °C but main coverture features switched to cyanobacterial groups of *Phormidium* spp. this observation is in accord with previous temperature experiments at Byers Peninsula (Velázquez et al. 2011). Conversely, the low growth rates displayed by many strains of cyanobacteria (Tang et al. 1997) probably exert that the experiment did not last enough to achieve differences in many cyanobacterial groups (e.g. *Phormidium* spp.). Even under optimal conditions for growth, these groups of phototrophs have slow doubling times relative to psychrophilic, eukaryotic algae. A small number of psychrophilic cyanobacteria have been identified (Nadeau and Castenholz 2000; Nadeau et al. 2001), but although these show a temperature optimum below most cyanobacteria and inhibition by moderately warm conditions, their growth rates are still quite low at temperatures below 10 °C (Vincent 2009). Beside, the measured increase of *Leptolyngbya* spp. coverture outlines a successful colonization strategy and ecosystem development, maybe by empty niche. However, psychrotolerance and slow growth in the cold is not successful in ephemeral environments such as melting snowbanks or in ecosystems where losses are substantial, for example as a result of strong grazing pressure or removal by advection (Convey and Stevens 2007). But under appropriate conditions (e.g. temperature) a cyanobacterial matrix might prompt further steps on ecological succession and consequently other organisms succeed.

This fact is also exemplified by the dominance of *Klebsormidium* sp. across all the microscopic enumerations. This taxon has a psychrophilic profile (Nagao and Uemura 2008). Although, those short-term assays address the importance of long-term records, in order to explain the communities´ growth and thermal strategies displayed by the different taxa of the assemblages. Antarctic freshwater habitats experience natural variability in environmental stresses. The magnitude of these variations generally is overweighing those linked with climate change, but the consequences of temperature augmentation on microbial benthic assemblages from maritime Antarctic freshwater ecosystems is that complex rather than simple responses are likely to be the leading principles.

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Capítulo 3/Chapter 3

Temperature effects on carbon and nitrogen metabolism in some Maritime Antarctic freshwater phototrophic communities

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ORIGINAL PAPER

Temperature effects on carbon and nitrogen metabolism in some Maritime Antarctic freshwater phototrophic communities

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ABSTRACT

Biofilms growing on ice and benthic mats are among the most conspicuous biological communities in Antarctic landscapes and harbour a high diversity of organisms. These communities are consortia that make important contributions to carbon and nitrogen input in non-marine Antarctic ecosystems. Here, we study the effect of increasing temperatures on the carbon and nitrogen metabolism of two benthic communities on Byers Peninsula (Livingston Island, Maritime Antarctica): a biofilm dominated by green algae growing on seasonal ice, and a land-based microbial mat composed mainly of cyanobacteria. Inorganic carbon photoassimilation, urea and nitrate uptake and N_2 fixation (acethylene reduction activity) rates were determined *in situ* in parallel at five different temperatures (0, 5, 10, 15, 25 °C) using thermostatic baths. The results for the cyanobacterial mat showed that photosynthesis and N_2 fixation responded positively to increased temperatures, but urea and NO_3^- uptake rates did not show a significant variation related to temperature. This microbial mat exhibit relatively low activity at 0°C whereas at higher temperatures (up to 15 °C), N_2 fixation rate increased significantly. Similarly, the maximum photosynthetic activity increased in parallel with temperature and showed no saturation up to 25 °C. In contrast, the ice biofilm displayed higher photosynthetic activity at 0°C than at the other temperatures assayed, and it showed elevated photoinhibition at warmer temperatures.

INTRODUCTION

Microorganisms that grow at low temperatures and nutrient availability, as well as cope with months of near complete darkness during winter, are often the dominant life forms in Antarctic freshwater ecosystems (Laybourn-Parry et al. 2002). These life forms have occupied the Continent since the Last Glacial Maximum (Convey and Stevens 2007). During the last century, a warming of Antarctic Peninsula has significantly exceeded that of the continental area, as reported by Steig and co-workers (2009). The potential effects of this warming on living organisms need more thorough investigation. From this perspective, knowing the eco-physiological consequences of rising temperatures for different species assemblages can help us to estimate the impact of warming trends on communities and therefore on ecosystems.

Cyanobacterial mats consist of a matrix of mucilage in which dominant cyanobacteria and other algal cells are embedded together with heterotrophic and chemoautotrophic microorganisms, sand grains and other inorganic and organic materials (de los Rios et al. 2004). Accordingly, the mats can be seen as multilayered structured ecosystems with relatively complex food webs. In Antarctica, these microbial communities usually dominate the benthic habitats of ponds and other shallow freshwater ecosystems, . They often account for almost all biological productivity in some polar environments (Vincent and Howard-Williams 2000). In Maritime Antarctica, microbial mats located in the catchment area of oligotrophic lakes are also an important allochthonous source of nutrients for the lakes via runoff (Toro et al. 2007). These communities are found where some liquid water is available during the thawing period every year and have a time span of several years. In this sense, they can be viewed as perennial communities. Other typical benthic communities are those dominated by green algae growing in or on the surface of seasonal ice, communities well known from alpine areas at temperate latitudes (Hoham et al. 2008; Hoham and Duval 2001), throughout the Antarctic region (Novis 2002; Ling and Seppelt 1998, 1993; Ling 1996), and from the Maritime Antarctic (Mataloni and Tesolín

1997). These communities only become conspicuous if the mean summer air temperature reaches or exceeds 0 °C (Ellis-Evans 1997). For this reason they are only evident when ice is present. When the ice thaws, they shift to the next stage of their life cycle. Accordingly, they are considered temporary communities. They persist as resistant forms until the next favourable period.

Different adaptive strategies are found in these two ecosystems. One strategy is to become a cold-condition specialist. Such specialists exhibit evolutionary adaptations for thriving at low temperatures. The phytoflagellate *Chlamydomonas,* and other green algae, are good examples of this strategy. Some of their strains posses a photosynthetic apparatus adapted to function under low-temperature conditions (Morgan et al. 1998). These so-called psychrophilic organisms can grow only at temperatures below 15 °C. In contrast, other groups of microorganisms that inhabit polar regions, including cyanobacteria, simply tolerate low temperatures and can grow despite the fact that such conditions put them far below their growth optimum (Tang et al. 1997). These organisms are called psychrotrophs.

Some limnological studies on microbial communities inhabiting shallow ecosystems have been performed in the South Shetland Islands and Antarctic Peninsula (Tell et al. 1995; Vinocur and Pizarro 2000), Continental Antarctica (Sabbe et al. 2004) and also in the Arctic (Bonilla et al. 2005). Few studies have investigated the effects of temperature variation *in situ* on microbial communities (Nadeau and Castenholz 2000; Pringault et al. 2001). The present work, a physiological study performed *in situ,* aims to compare the carbon and nitrogen metabolism of a psychrophilic biofilm and a psychrotolerant cyanobacterial mat growing in a mild Antarctic region where air temperature exceeds 0 °C in summer.

MATERIALS AND METHODS

Study site

Byers Peninsula (Livingston Island, South Shetland Islands), the largest ice-free area in Maritime Antarctica, supports very abundant and diverse aquatic ecosystems (Toro et al. 2007). The area is located at the west end of Livingston Island (62° 34'35" - 62°40'35" S and 60°54'14" - 61°13'07" W) (Figure 1). The peninsula, 60.6 km² in area, is largely free of ice and snow cover during almost the entire austral summer season (December to March). The low-lying areas of the peninsula favour the retention of surface water and include temporary waterlogged areas, lakes and pools (López-Martínez et al. 1996). The microbial mat studied here grew on the plateau of the peninsula. In contrast, the ice biofilm grew on the seasonally accumulated icy snow at the South Beaches (Figure 1).

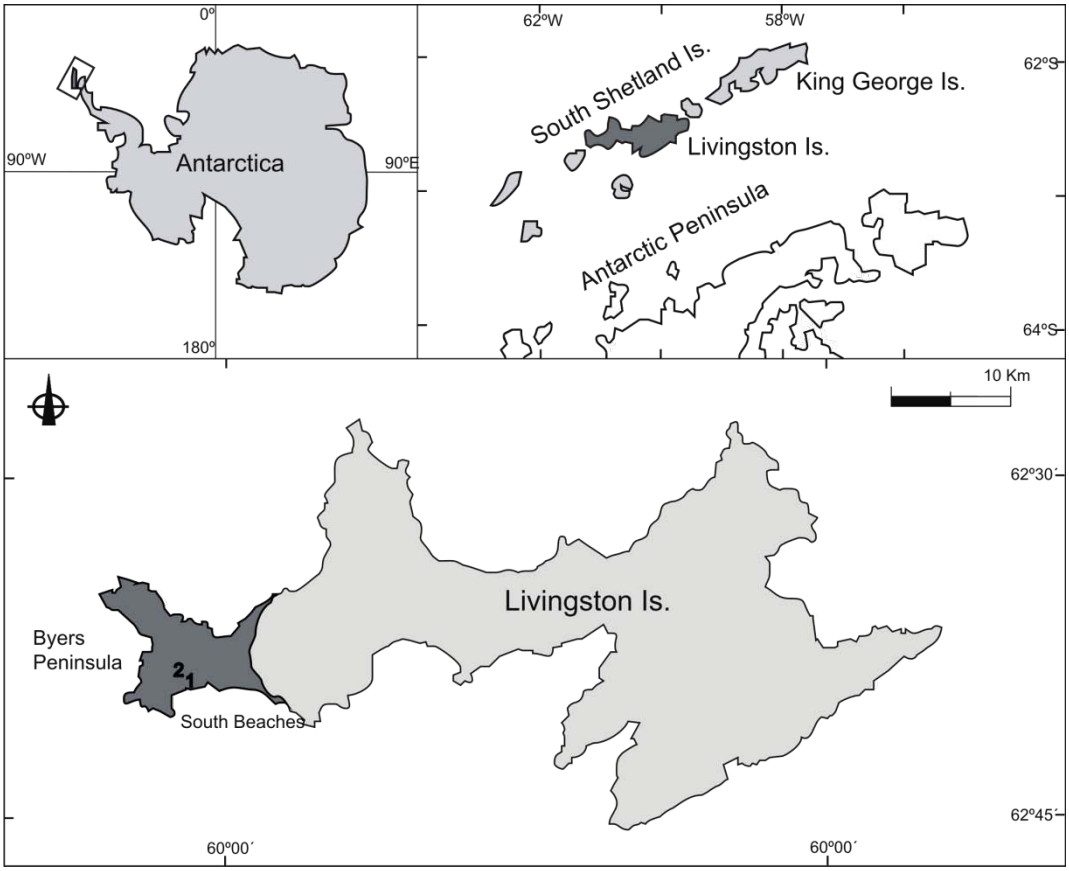

Figure 1. Location of Byers Peninsula on Livingston Island in the South Shetland Islands of Maritime Antarctica. (1) Site location of Ice Biofilm at South Beaches (5m asl). (2) Site location of Purple Mat (70m asl).

Microscopic study

Samples for microscopic observations and taxonomic determinations were taken with 15 or 22 mm inner–diameter metal core samplers. Stones and gross-sized sediments were removed, and the samples were kept frozen at -20 °C for microscopic determination. Observations were carried out by using both bright field and epifluorescence microscopy at the field camp immediately after collection. The samples were subsequently sent to the laboratory in Spain for further microscopic where microscopic study.

The composition of the phototrophic assemblages within the communities was studied with fresh samples, using two different fluorescence filters and Nomarsky interference microscopy. For each microscopic field, observations were done under white illumination and using an Olympus® blue filter set (EF 400-490 nm, DM 570, FB 590) where chlorophyll *a* was excited, whereas for excitation of cyanobacterial phycobiliproteins an Olympus® green filter set (EF 530-545 nm, DM 570, FB 590) was used.

Although recent taxonomic literature of the taxonomy for polar cyanobacteria was consulted (Komarek and Anagnostidis 1999, 2005), the Broady and Kibblewhite (Broady and Kibblewhite 1991) morphotype definitions were followed to provide simple morphological assignments. Ling and Seppelt (1998), Duval et al. (1999) and Mataloni and Tesolín (Mataloni and Tesolín 1997) were also used for taxonomical identification of the biofilm community.

Experimental setting and analytical methods

The purpose of our experimental design was to allow physiological comparisons between two distinct communities found on Byers Peninsula: an Ice Biofilm (IB) growing on the accumulated seasonal icy snow on the southern beaches of Byers Peninsula and dominated mainly by green algae, and a microbial mat, the Purple Mat (PM), a well-developed cyanobacterial consortium growing on a shallow pond and highly stable across years.

Experiments were carried out *in situ* during the last week of January with material collected on the day of the experiment. Samples of both communities were obtained using a metal corer of 22 mm inner diameter. For each of the assayed temperature and light conditions, three replicates of each community were placed inside Whirl-pak® bags (60 ml. Nasco) filled with 10 ml of water (pond water or melted snow, for PM and IB, respectively) filtered through GF/F glass fibre filters (Whatman).

Uptake assays for inorganic carbon, nitrate, urea and nitrogenase activity were performed by means of the stable isotope technique (see below for further details) at the camp site using 5 thermostatic baths at temperatures of 0, 5, 10, 15 and 25 °C. All the incubations were performed for two hours under natural-light exposure. The temperatures logged on the mat surface where samples were collected and during the week the study was performed were from 0 °C to 10 °C for PM and from 1 °C to 4 °C for IB.

Different irradiance conditions were also assayed in the determinations of inorganic carbon uptake. The different light intensities for this assay were achieved by using neutral filters. The resulting light levels during incubations were 100, 63, 24, 15, 7.8, 4.5, 1.5, 0.8, 0.5 and 0% of incident light. The transmitted light was measured with a 2π quantum sensor (Li-Cor, LI-192) connected to a datalogger (Li- Cor, LI-1000). After experiments were finished, samples were washed to remove the non-fixed isotopes and transported frozen to Spain.

In the laboratory, cores were weighed and dried at 60°C for 24 hours. After grinding, the isotopic enrichment of samples relative to non-treated controls was determined using an IRMS Micromass-Isochrom mass spectrometer. The particulate organic carbon (POC) and nitrogen (PON) content of both communities were measured from the dried samples by using an Elemental Analyser LECO CHNS-932.

For chlorophyll *a* (Chl-*a*) analyses, the lipophilic pigments were extracted twice in dark conditions with 90% aqueous methanol for 24 hours and 1 hour consecutively. the concentration of extracted pigments was then measured in a diode array

spectrophotometer (MultiSpec-151, Shimadzu). Absorbance was measured at two wavelengths: 750 nm, as a turbidity reference, and 665 nm, as a main peak of Chl-*a* absorbance. The Chl-*a* concentration values were expressed in µg Chl-*a* cm^{-2} determined using the coefficient of Marker (Marker et al. 1980).

Inorganic carbon uptake

Photosynthetic carbon assimilation was measured as ^{13}C (98% ^{13}C. Isotec) incorporation in samples, with $NaH^{13}CO_3$ as a tracer (Ariosa et al. 2006) from a stock solution of 1 mgC ml^{-1}. The dissolved inorganic carbon (DIC) was calculated from water alkalinity (by considering pH and temperature), measured after titration with HCl using a pH shift indicator (phenolphthalein) of equivalence endpoint pH. Incubations were performed in thermostatic baths at the temperatures and light conditions mentioned above. The incubations were started by adding the ^{13}C tracer to the bags at an approximate concentration of 10% of the natural concentration of ^{12}C. After two hours, the incubations were stopped by adding 1 ml of 1N HCl per bag to exclude non assimilated ^{13}C to the atmosphere in $^{13}CO_2$ form. During this step, the bags were kept open. Then, 1 ml of 1N NaOH was added to neutralise HCl, and samples were rinsed immediately with GF/F-filtered pond water. Samples were dried, frozen and transported to the laboratory.

Nitrate and urea uptake

Nitrate and urea uptake rates were measured by using urea-^{15}N (98% ^{15}N, CIL) and $K^{15}NO_3$ (99.9% ^{15}N, Isotec). The stock solutions used in the assays were 30 and 4 µgN ml^{-1}, respectively. From these stocks, the appropriate volume was added to bags to reach 10% of the natural concentration of the light isotope, as measured the previous year in the same area. The urea was not measured in the environment and was added at the same concentration as DIN.

Nitrogenase activity

The rates of N_2 fixation (nitrogenase activity) were determined as the acetylene reduction activity (ARA) after collecting the biomass, as described by Fernández-Valiente, et al. (2001), with the same light intensity as the PvsI curves described above. In brief, two cores of 22 mm inner diameter from each community were immersed in 100 ml of GF/F filtered surrounding water in 250 ml flat tissue culture flasks (IWAKI, Asahi Glass Co., Ltd.) sealed with rubber stoppers. The experiments were run in triplicates. To each flask, pure acetylene obtained from calcium carbide was added at 10% (v/v) concentration. After four hours of incubation, ethylene produced by the nitrogenase from acetylene reduction was collected by extracting air from the incubation flasks and transferred to 10 ml vacuum tubes (Vacutainer). The tubes were wrapped with film and stored at 4°C for shipping to the laboratory. There, ethylene concentration was determined by a gas-chromatograph (Shimadzu GC-8A) equipped with a flame ionisation detector using a Porapak N80/100 column.

Mathematical and statistical analysis

Photosynthesis *vs.* irradiance curves for Purple Mat (PM) were fitted to the Platt photosynthesis model (Platt et al. 1980), which follows a hyperbolic tangent equation at each temperature, using Sigmaplot software (Systat Software Inc.). Here, P_s is defined as the estimated maximum photosynthetic rate, α as the initial slope of the curve (a measure of photosystems efficiency) and E as the irradiance in each interval of the curve. The ice biofilm (IB) data did not fit this equation. Our experimental data unexpectedly showed extremely quick saturation of photosynthesis and photoinhibition, even under very low irradiances. In this case, the experimental data were fit to the exponential difference equation proposed by Ritchie (2008). Here, plotted values were the values predicted by least square fitting. Correlation was expressed using the Pearson correlation coefficient. To compare the different assays for both communities, Q_{10} values were calculated. The Q_{10} value obtained over the range from 0 ° to 25 °C was used as a standard indicator of the

cold adaptation of the physiological activities measured. This value reflects activity variation within a given temperature increment (Vincent 2000b) as follows:

$$Q_{10} = (k_2 / k_1)^{10/(t_2 - t_1)}$$

Where the expression (k_2/k_1) is the activity ratio at both temperatures assayed and (t_2-t_1) is the difference in temperature. If appropriate, a paired t-test or ANOVA with the Tukey post-hoc test were used to compare the means of different treatments. SPSS Statistics 17.0 (SPSS Inc., 2008) and Sigmaplot 11.0 (Systat Software Inc.) were used for all statistical procedures.

RESULTS

Phototrophic community structure

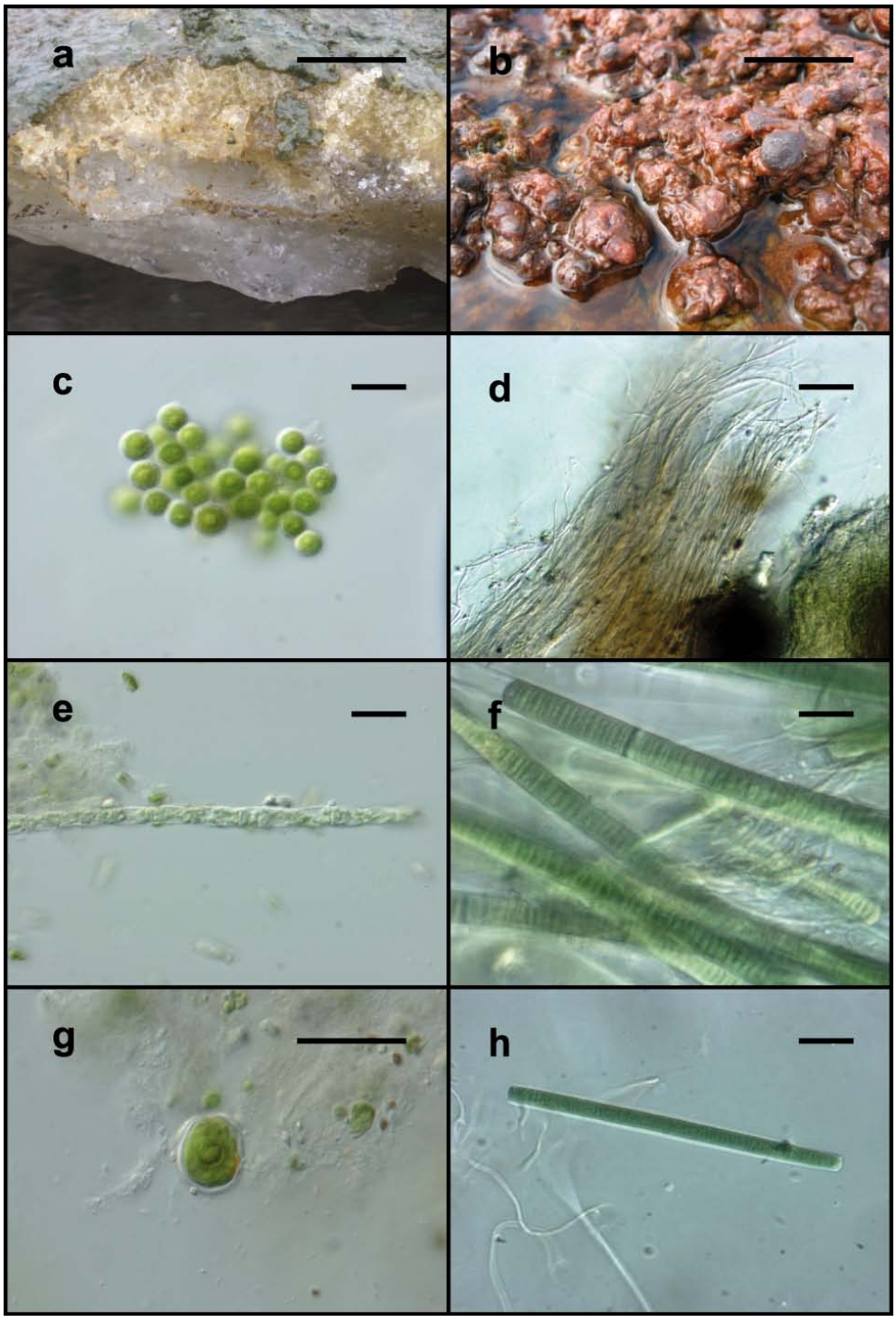

Figure 2. Microphotographs of the communities assayed. Scale bar represents 10 µm in a, c, e, g and 1 cm in a, b. (a, c, e, g) Ice Biofilm. (b, d, f, h) Purple Mat. (a) Macroscopic mat structure of IB. (b) Macroscopic mat structure of PM. (c) Cryobiontic *Chlorophyta*. (d) PM matrix. (e) *Klebsormidium* sp. (g) *Chlamydomonas* sp. (f, h) cf. *Phormidium* spp.

The two communities studied differed macroscopically. The ice biofilm (IB) was dark green at the surface, thinner (1-1.5 mm) and had a gelatinous texture (Figure 2a). In contrast, the microbial mat (PM) showed a purple-greenish surface colour, was 3-4 mm in thickness and had a rugged surface (Figure 2b). The substrates for the communities are also different. IB occurred in seasonal ice from precipitation, whereas PM occurred in semi-permanent shallow ponds.

Microscopic observations showed marked differences in composition among the communities. The matrix of PM consisted of two thin cyanobacteria of the morphotypes I (1 μm in diameter) and J (3 μm in diameter) *sensu* Broady and Kibblewhite (1991) (Figure 2d). The work of these authors is based on the Oscillatorian diversity of Ross Island and Southern Victoria Land in Continental Antarctica. Intermixed with the matrix filaments were many unicellular cyanobacteria (1.5 μm in diameter). Thicker cyanobacteria from morphotype C (5.5-6 μm in diameter) were also present within the mat. According to Anagnostidis and Komarek (1988) all these morphotypes can be assigned to different species of the genus *Phormidium* (Broady and Kibblewhite 1991), but morphotype I presumably belonged to genus *Leptolyngbya*. Other filamentous cyanobacteria present in the surface layer included different *Phormidium* species assigned to morphotypes B and K (4-5 μm in diameter) (Figure 2h) and morphotype E (11 μm in diameter), which were also abundant (Figure 2f). Furthermore, an unidentified filamentous cyanobacterium with a dark thick sheath (18 μm in diameter) was observed profusely at the mat surface layer. Cyanobacterial cells surrounded by thick sheaths and 5.5 μm in diameter were observed as well. These cyanobacteria probably belong to the family *Phormidiaceae* because they exhibited a thick, lamellated and coloured sheath. Diatoms were also present in this mat but at low densities. On the other hand, abundant microcolonies of *Nostoc* sp. appeared in the deepest layer. In contrast, the IB photosynthetic community was dominated by forms of the genera *Chloromonas sp.*, *Chlamydomonas sp.* and *Klebsormidium sp.* (green algae) (Figure 2c, 1g and 1e). Moreover, some filamentous cyanobacteria presumably of the

genus *Phormidium* (assigned to the morphotypes B and K, 4-5 µm in diameter) and thinner filamentous cyanobacteria of the genus *Leptolyngbya* were found within the community.

The values of dry weight per unit surface area were very similar in both communities investigated (Table 1). Results for chemical composition were as follows, PM showed slightly higher C content per dry weight than IB (t-test: P<0.001) (Table 1). In contrast, N content, as a fraction of dry weight, was almost twofold higher in IB than in PM (t-test: P<0.001). This difference in N content resulted in much higher C/N ratios for the PM community than for the IB community (ANOVA: P<0.001). The chlorophyll-*a* (Chl-*a*) content per surface area did not show statistical differences among communities (ANOVA: p-value=0.381). Both communities showed differences in their carbon and nitrogen isotopic composition, and this result suggests some metabolic differences. Hence, although the ^{13}C natural abundance was significantly more negative in PM (ANOVA: p-value<0.001), the ^{15}N isotopic signatures were very similar for both communities (ANOVA: p-value=0.809).

Table 1. Biochemical characterization of the surveyed microbial communities. Dry weight (DW), dissolved inorganic nitrogen (DIN) and dissolved inorganic carbon (DIC). Data are averages of three or more replicates for most analysis. (*) community-overlying water for PM and snow in the IB case.

Variable	Purple Mat (PM)	Ice Biofilm (IB)
DW (mg cm^{-2})	153±78	151±99
Carbon (mg gdw^{-1})	228.4±3.4	210±2
Nitrogen (mg gdw^{-1})	18.3±0.2	35.9±0.97
C/N	12.5±0.1	5.9±0.2
Chlorophyll *a* (µg cm^{-2})	20.0±3.5	16.1±5.9
DIN (µgN l^{-1})*	50	162
DIC (mgC l^{-1})*	1.44	1.88
$\delta^{13}C_{PDB}$	-12.1±0.6	-9.3±0.3
$\delta^{15}N_{air}$	2.5±0.4	2.4±0.8

Photosynthetic features under different temperatures

Because the experiments were conducted under natural conditions on different days for both communities, the irradiance regimes were different for both assayed communities. Maximum irradiance values during the incubations experiments were 380 µE m^{-2}s^{-1} and 1852 µE m^{-2}s^{-1} for the purple mat (PM) and the ice biofilm (IB) respectively.

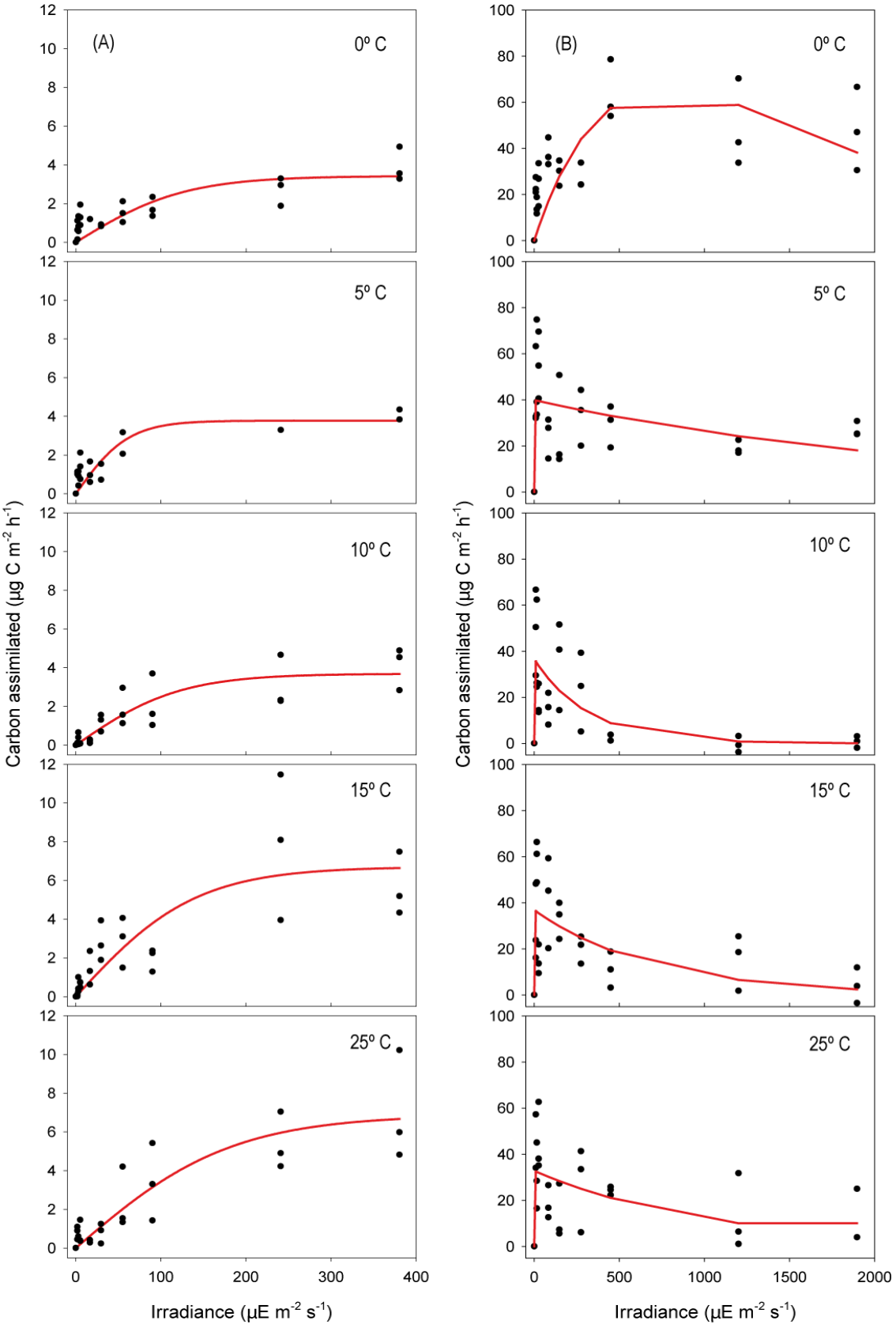

Figure 3. Photosynthesis vs. Irradiance curves for each incubation temperature in both communities studied. (a) Purple mat (PM), (b) Ice biofilm (IB). Mean irradiance values during the assays were 350 µE $m^{-2}s^{-1}$ for the Purple mat and 1850 µE $m^{-2}s^{-1}$ for the Ice biofilm. The line shown for PM shows the fitted values obtained using the Platt et al. model (1980). The line shown for IB represents smoothing values of the data.

Carbon assimilation in PM increased with temperature (Figure 3a). Indeed, at 25 °C the maximum photosynthetic rates were twofold higher than at 0 °C. The PvsI curves for this community were, however, similar from 0 °C to 10 °C and showed significantly lower values of maximum estimated photosynthesis (P_s) compared to those found at 15 ° and 25 °C (Figure 3a). Photoinhibition was not found at any temperature at the irradiances assayed (Figure 3a). Photosynthetic efficiency values (α) were all very similar at the different temperatures assayed and ranged between 0.01 and 0.02 μg C $cm^{-2}h^{-1}$ (μE m^{-2} $s^{-1})^{-1}$ (Table 2). Results for this community data indicate low rates of photosynthesis at lower temperatures. Increases in temperature were accompanied by increased rates of carbon assimilation, ca. a twofold increase (Table 2). In this sense, Q_{10} values calculated between 0 and to 25 °C for each carbon uptake (P_s) assay exhibited a relevant temperature effect, with Q_{10} =1.8.

Table 2. Estimated photosynthetic parameters from both communities. Maximum photosynthesis (P_s) values are in μg C $cm^{-2}h^{-1}$. Photosynthetic efficiency (α) units are μg C$cm^{-2}h^{-1}$ (μE m^{-2} $s^{-1})^{-1}$). These values were fitted to Platt et al. (1980) model for each photosynthesis versus irradiance curve at given temperature for PM. IB data were fitted to an exponential difference equation proposed by Ritchie (2008). Chlorophyll-specific photosynthetic rates (Ps^{Chl}) are measured as μg C (μg Chla $h)^{-1}$. r^2 is identified by the fitted boundary of the models.

Community	Temperature (°C)	α	P_s	Ps^{Chl}	r^2	Ek
PM	0	0.010	3.42	0.17	0.57	342.0
	5	0.023	3.77	0.19	0.61	163.9
	10	0.011	3.69	0.18	0.78	335.5
	15	0.018	6.70	0.33	0.67	372.2
	25	0.014	6.88	0.34	0.78	491.4
IB	0	0.23	65.5	4.07	0.71	284.8
	5	80.1	39.8	2.47	0.53	0.50
	10	77.8	36.3	2.25	0.70	0.46
	15	74.1	36.7	2.19	0.63	0.49
	25	71.0	32.7	2.03	0.51	0.46

The different PvsI curves obtained as a function of the temperature in the ice biofilm (IB; Figure 3b) were fitted to the exponential difference equation proposed by Ritchie (2008). This formulation accounts for the photoinhibition of the system more

accurately than other previously proposed models (Platt et al. 1980; Jassby and Platt 1976; Webb et al. 1974; Frenette et al. 1993). Probably owing to the extreme thinness and heterogeneous composition of this community, the data dispersion was quite high.. Uncertainty in the estimated parameters could be excessively high in some cases. Nevertheless, photoinhibition was evident at 0 °C, a naturally-occurring temperature for organisms growing on ice. The P_s values increased with irradiance (Figure 3b) up to the highest level and then decreased at the highest irradiance. The PvsI curves at 5 °, 10 °, 15 ° and 25 °C were likewise very similar and exhibited clear photoinhibition effects at very low irradiance (Figure 3b). At higher temperatures, P_s values were very similar (an average of 36.3 µg C cm^{-2} h^{-1} ±2.9) and they took place under very low irradiance (between 15 and 26 µEm^{-2} s^{-1}, values extracted from the raw data), followed by an intense photoinhibition, except in the case of the 0 °C assay. Those P_s values were one order of magnitude higher than those found in the PM, even when expressed as specific activity (µg C (µg Chl *a* h)$^{-1}$). Moreover, in IB, there were no relevant differences in C uptake between low and higher temperature treatments, and the Q_{10} for P_s between 0° and 25°C was 0.5, indicating a lower activity rate.

The parameters derived from the light-dependent curves difference significantly between communities. This result indicates a relative difference in photosynthetic light efficiency at low irradiances (Figure 3 and Table 2). The irradiance of saturation (E_k) values (defined as P_s/α) obtained in PM were somewhat similar at all the temperatures assayed (Table 2), averaging 341±117 µE $m^{-2}s^{-1}$. In contrast, E_k for IB averaged 0.48±0.02 µE $m^{-2}s^{-1}$ (excluding the E_k value for 0 °C, 284.8 µE $m^{-2}s^{-1}$). For the photosynthetic efficiency (α), IB shows higher values, reflected in the near vertical slopes of the PvsI curves. Owing to the strong differences in natural light conditions when the experiments were carried out, these results should be interpreted cautiously.

Chlorophyll-specific photosynthetic rates (Ps^{Chl}) were one order of magnitude higher in IB compared with PM (Table 2), ranging respectively 2.06- 4.07 µgC (µg Chl*a* h)$^{-1}$ and 0.17- 0.34 µgC (µg Chl*a* h)$^{-1}$. These differences parallel those observed for P_s.

Temperature dependence of nitrogenase activity

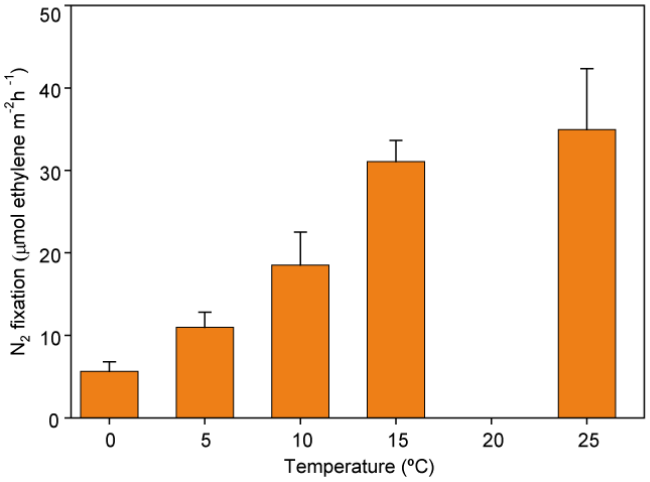

Figure 4. Results of the acetylene reduction assays (nitrogenase activity) at each assayed incubation temperature for PM. The assay was performed during the afternoon. Irradiance during the experiment ranged from 255 to 755 µE $m^{-2}s^{-1}$. Data are means ±SD of three replicates.

Significant acetylene reduction activity (ARA) was found only in the PM. Likewise, heterocystous N_2-fixing cyanobacteria (Nostocales) were only found in this community, whereas non-heterocystous cyanobacteria, also thought to be potentially N_2-fixing, were a minority in IB. Our results indicated that N_2-fixation depended strongly on temperature (Figure 4), with a Q_{10} value defined between 0° and 25 °C of 5.8. ARA increased up to 15 °C, showing no significant differences from the activity measured at 25 °C (ANOVA: p-value=0.823), on the contrary, significant differences were found when the values from 0° to 10 °C were compared to those of 15° and 25 °C (ANOVA: p-value<0.001). On average, N_2-fixation measured at 25 °C was 7-fold higher than that found at 0 °C.

Urea and nitrate temperature-dependent uptake

Trends in the assimilation of urea and nitrate at different temperatures under natural light were assayed in parallel with PvsI incubations (see Materials and Methods). Uptake rates of nitrogen compounds for both communities suggested that nitrogen uptake was independent of temperature regime (paired t-test; p-value<0.0001 and p-value<0.0001 for

urea and nitrate uptake respectively in PM; and p-value<0.0001 and p-value=0.0002 for urea and nitrate uptake respectively in IB) (Figure 5). As shown in Figure 5, urea and nitrate uptake ratios were almost one order of magnitude lower in PM than in IB at each temperature evaluated.

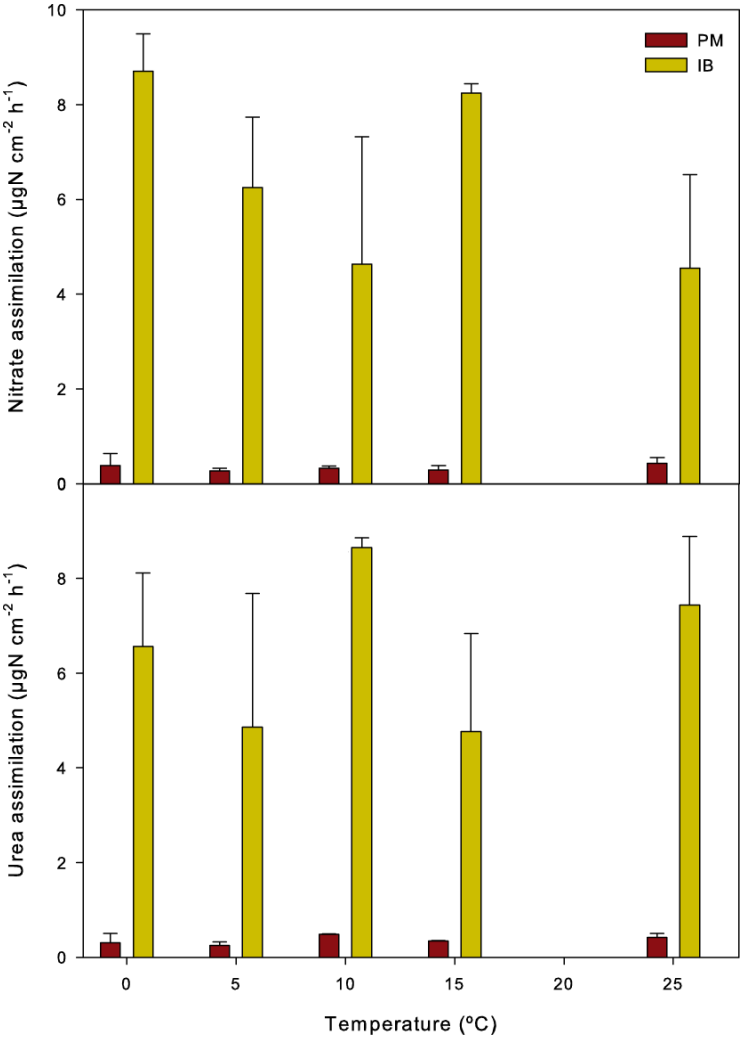

Figure 5. Nitrate and urea uptake at different temperatures expressed per unit surface area in two microbial communities at Byers Peninsula, Livingston Island (Antarctica). Data are means ±SD of three replicates

DISCUSSION

Maritime Antarctica is one of the regions of the Earth where climate change is most pronounced (Quayle et al. 2002). Nevertheless, studies of the effects of temperature change on the physiological activity of aquatic organisms from this region are scarce. Our research addressed the need for such additional studies. We sought to analyse and to compare the short-term effects of temperature shifts on carbon and nitrogen metabolism in two Antarctic microbial communities, one dominated by cyanobacteria (Purple Mat, PM) growing on a shallow pond; and the other dominated by chlorophytes (Ice Biofilm, IB) growing over annual ice. The experiments were run without preincubation periods in order to measure the organisms´ physiological status at the moment of the experiment. We chose this approach because experiments including previous acclimation can produce strain-dependent results (Hoham et al. 2008). Such results can be difficult to interpret for natural mixed communities.

PM is a perennial mat-forming community that has remained viable for several years (personal observation). It is entirely exposed to fluctuating physical conditions at daily and seasonal scales as temperature or liquid water availability vary, but it is covered by snow and ice from fall until spring. The main components of PM, cyanobacteria, are considered to be psychrotrophs instead of psychrophiles. Psychrotrophy has been already described by Mueller et al. (2005) for other cyanobacterial mats in the High Arctic. In contrast, Nadeau and Castenholz (2000) isolated a cyanobacterium from a pond on the McMurdo Ice Shelf (Antarctica) and found that this organism appeared to be psychrophilic. Their cyanobacterium exhibited higher physiological activity at lower temperatures characteristic of different polar ecosystems (Taton et al. 2003). Nevertheless truly psychrophilic strategies would not be suitable in shallow-water ecosystems in the non-continental region of Antarctica, where summer water temperatures very often reach 15 °C. In contrast, IB is a seasonal community that disappears after the ice melt and emerged only under particular conditions. Indeed, on Byers Peninsula this type of

community developed completely only once in 6 summers. The environmental conditions in late spring, when this community grows, are quite stable with similarly cold temperatures provided by the ice-melt-water interphase. Chlorococcales and Volvocales are the primary constituent taxa of IB. This cryosestonic community has some taxa in common with the assemblages previously described by Mataloni and Tesolín (Mataloni and Tesolín 1997). The organisms in those assemblages are generally considered psychrophilic. As long as the ice remains, this community enjoy optimal conditions. When temperatures rise, cells differentiate into resistant spores (Hoham et al. 2008).

Our main hypothesis about the differing strategies found in the two types of communities agrees well with the PvsI curves obtained. In fact, these curves show completely different patterns. P_s values for PM exhibit a remarkable increase in response to increasing temperatures up to 15 °C. In contrast, the values of this parameter in IB decreased dramatically from 5 °C upwards. A possible explanation of these observations is that the cold-tolerant organisms forming the PM community survive under suboptimal temperatures and can thrive under a broad range of conditions, even though their photosynthetic activity slows down at colder temperatures. In contrast, the organisms inhabiting the IB are stenothermal, displaying an optimal temperature for maximum photosynthesis close to 0 °C; indeed, at temperatures slightly above 0 °C the P_s in this biofilm is markedly reduced by 45%. Nevertheless, at least in the short term, temperatures as high as 25 °C did not inhibit photosynthetic activity under moderate photon flux rates. Differences between the two communities are even more evident from a comparison of the Q_{10} values. Although IB shows a negative Q_{10} value, PM shows a positive (1.8) value. These results clearly indicate that the IB organisms are well suited to cold temperatures, whereas the PM organisms do best under warmer conditions. Other Antarctic communities dominated by Oscillatoriaceae exhibited Q_{10} values between 2.3 and 1.7 (Goldman et al. 1963; Vincent and Howard-Williams 1986). A Q_{10} value of 2 is the assumed theoretical value for temperature-dependent kinetic effects (Falkowski and Raven 1997)

for non-cold-adapted organisms. So far as we know, no other findings on this topic for Antarctic benthic communities composed of chlorophytes have been published to date. The results for photosynthetic efficiency (α), indicates higher values in the IB community than in PM. This finding suggests that the photosynthetic mechanism of chlorophytes captures light more efficiently than does that of cyanobacteria (Table 2). Furthermore, this higher photosynthetic efficiency might explain the greater amount of photoinhibition exhibited by the IB community with increasing temperature (Figure 3b). If the majority of the IB organisms are truly psychrophilic, then they should be heat stressed under high temperatures. Photoinhibition in vascular plants is known to be greater under high light regimes. Under these circumstances, the xanthophyll cycle plays a crucial role in protecting PSII against heat induced photoinhibition (Yin et al. 2010). However, our findings seem to disagree with the results obtained by Remias et al. (2005) for the snow algae communities from a high mountain environment. That study found no photoinhibition up to 1800 μE m^{-2} s^{-1}. Furthermore, the highest photosynthetic rate found was in higher temperatures. In contrast, other studies, e. g., Ling and Seppelt (1998, 1993) found that Antarctic snow algae were killed by temperatures above 10 °C.

Although the IB community showed no detectable nitrogenase activity, the rates of N_2-fixation in the PM community were in the same range as that suggested by previously published data from the area (Fernández-Valiente et al. 2007). Indeed, the dominance of heterocystous cyanobacteria (Nostocales) in the PM mat was confirmed by microscopic observation, even though these organisms were absent from the IB biofilm. The temperature dependence of N_2-fixation was higher for Byers Peninsula than for other parts of Antarctica (e. g., East Antarctica) (Howard-Williams et al. 1989). This difference is evident from the relative Q_{10} values, despite the fact that the analyses involved different types of communities (streams vs. ponds). The dependence of N_2-fixation on temperature has been also described in tropical oceans (Staal et al. 2003). The temperature

independence of N uptake in both communities indicates that even if one of them is psychrophilic for a given metabolic activity, the same is not necessarily the case for others.

The low fixation rates of both inorganic carbon and N_2 observed in the PM community at around 5 °C, added to its sensitivity to temperature changes, suggest that this cyanobacteria-based community accumulates biomass during the brief periods of warmer summer temperatures. This strategy contrasts notably with that observed for the IB biofilm. The latter community exhibits explosive growth when conditions are optimum. The extremely high inorganic C and combined N uptake rates observed during colder periods permit extremely rapid growth rates in this cold-adapted community (authors' observations). Indeed, during this short period of appropriate conditions the IB community can produce as much biomass as the PM, measured by carbon and chlorophyll *a* content. But, in contrast to the PM mat, the development of this biofilm is greatly restricted to short time periods and continues only until the ice thaws.

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Capítulo 4/Chapter 4

Seasonal dynamic of an Antarctic microbial mat: physiological and ecological interactions

"Me figuro que si Dante hubiera visto nuestro barco en los momentos más difíciles, se le habría ocurrido una buena idea para describir otro circulo del infierno, aunque no habría sabido qué hacer con una pandilla de individuos tan alegre y atrevida como la nuestra"

Raymond Priestley. El peor viaje del Mundo.

ABSTRACT

A study of the physiological characteristics and relationships between the different compartments of a microbial mat was carried out during summer of 2006-07 at Byers Peninsula (Maritime Antarctica). This survey of *Cyanobacteria*, *Chlorophyte*, metazoan and fungal biomass is aimed to identify the relationship between organisms within microbial mats, estimating changes in biomass, carbon flows, some of their physiological activities (primary production, secondary production, N uptake, N_2-fixation) and the relationship between the different levels in a given physical environment. The main hypothesis is that the benthic mat community is not limited in both biomass and productivity by inorganic carbon supply but mainly by trophic relationships and recycling processes of C budget stored at the different compartments of the community. The results in combination with the chemical analysis of mat overlaying water and trophic network analysis indicate that neither carbon nor other nutrients were likely to be limiting. This allowed a more detailed analysis of the organization and biological responses of the microbial mat along the flourishing season. The results show an equilibrium stage of the community and many matching events that relate some environmental factors with biological features. Besides, in the proposed model, recycling processes within the community play a key role in C dynamics and nutrients turnover.

INTRODUCTION

Many lakes and ponds melt out over Polar landscapes each summer where the organisms form highly pigmented and structured microbial mats and cover the substrate (Vincent 2000c). The mats are consortia of many organisms as bacteria, diatoms, green algae, fungi, protozoa, metazoan, and associated viruses to all groups, but their main biomass constituents are mainly Oscillatorian cyanobacteria. Cyanobacteria have often been recorded as the dominant phototrophs in Antarctic terrestrial and freshwater ecosystems (Taton et al. 2003).

Microbial mats are typically thought of as low resources ecosystems in which the photosynthetic communities are severely constrained by nutrient supply. In that way, many studies have been carried out all around Antarctic and Arctic ecosystems, drawing attention to the supposed ultraoligotrophic characteristics of the flowing water, but those characteristics differ enormously from the interstitial water within the benthic community in which nutrients may reach high concentrations (Tang et al. 1997). In many deeper Antarctic water bodies, nutrient enrichment bioassays have confirmed the strong limitation of their phototrophic consortia by phosphorus and/or nitrogen availability (Bonilla et al. 2005).As one of the main microbiological features in Antarctic limnetic ecosystems, those benthic communities appear to be the major source of organic carbon into the watershed area of lakes as well as benthic food webs and can play a leading role in the production dynamics of the overall ecosystem (Burkholder and Wetzel 1989). Opposed to planktonic phototrophic communities, the benthic prototrophs may be subjected to different controlling factors (Rautio and Vincent 2006). In shallow lakes and ponds of both polar regions, cyanobacterial mats may achieve high standing stocks and often dominate overall ecosystems productivity (Vincent 2000c; Hawes and Schwarz 1999; Hodgson et al. 2004), although this may represent the gradual accumulation of slow-growing species over many seasons (Vincent et al. 2000a).

In addition to nutrient constraints, microbiological communities of Polar Regions are subjected to large variations in light availability, from total winter darkness to continuous light in summer, coupled with long periods of freezing and a short growing season (Ellis-Evans and Walton 1990). During summer ice-out conditions, the benthic mats can be abruptly exposed to high level of PAR, fluctuating nutrients inputs or a wide range of temperature regime. The communities therefore require a broad set of strategies embracing those alternating conditions (Paerl et al. 2000). Previous studies on high latitude microbial mats have shown that they can be vertically structured to self organize and optimize their physical and chemical resources (Vincent et al. 1993). The resultant optimization and connections of growth rates are also likely to minimize nutrient requirements and the potential for nutrient limitation.

Many studies have been carried out on microbial communities in the Antarctic, due to the major and often dominant contribution of microbes to total ecosystem biomass, biodiversity, nutrient cycling and energy flow (Vincent 2000b). However, complete growing season studies, as this one presented here, are scarce. As most ecosystems on the Biosphere, microbial communities show ecological succession in response of physical, chemical and biological factors. Microbial mats are composed by a cohort of taxa with different trophic strategies and specialization in diverse niches. Cyanobacterial microbial mats are thought to be responsible for much of the primary production in extreme polar environments (Tang et al. 1997; Vincent et al. 2000b), and such communities are often characterized by increased nutrient supply relative to the overlying water (Bonilla et al. 2005). A detailed monitoring of the dynamics of those communities will show a crucial part of the ecology of the Antarctic microbial mats.

In order to understand the processes that sustain the microbial mat ecosystems and to assess the role of each process, it is necessary to include a trophic web perspective, trying to illustrate quantitatively the matter flow connecting the community components. However, it is almost impossible to obtain all these flows from field observations because

of methodological problems estimating actual figures (e. g. bacterial respiration dissolved organic carbon excretion and so on). A modelling approach, as Ecological Network analysis (ENA), can overcome this difficulty. So, from conceptual models, the measurements of different activities that relate organism relationships help us to weigh the importance of every edge of the network accomplished within, with the aim of monitoring and modelling the community dynamic showing the holistic characters that rise from ecological network approaches. In that way, descriptions by using the indexes of Ecological Network Analysis (Ulanowicz 2004) will highlight the consequences on emerging properties derived from the proposed model.

Here, a cyanobacterial based community growing on an Antarctic wetland is investigated from spring to late summer of the 2006-07 sampling season. The community investigated was chosen from a wetland at Byers Peninsula (South Shetland Islands) which is at the northern limit of the Antarctic Peninsula region and a key monitoring spot for climate change due to its high biodiversity, spreading over a wide diversity of freshwater bodies. In fact, Rautio and co-workers (2008) pointed it as one of the main biodiversity hot spot in Maritime Antarctica. The main objectives in the present study are: first, to describe the different features within the mat trophic web and how they are related one each other. Second, to determine whether there is any nutrient limitation in the benthic microbial mats of Byers Peninsula. In this point, our main hypothesis is that the growth of benthic mat community is not limited in both biomass and productivity by inorganic carbon supply but mainly by trophic relationships and predation processes. This aspect is investigated via modelling the flows between the different compartments within the community. Moreover, the displayed model shows how the community adapts its internal characteristics to assume changes in the physical and chemical constrains, in order to maintain the whole system homeostasis through several achievable scenarios. This allows a more detailed analysis of the organization and biological responses of the microbial mat along the flourishing season.

MATERIAL AND METHODS

Case study and general features

The climatic conditions on Byers Peninsula are maritime (Vincent, 1988) with meteorological records at the plateau of the Peninsula, indicating mean annual temperatures of -2.1 °C, mean summer temperatures 1.5 °C, and an annual precipitation rate of 800 mm (recorded from Juan Carlos I Station, Hurd Peninsula. Livingston Island) (Bañón 2001). As a result of these conditions, Byers Peninsula is snow-covered for the majority of the year, but is almost completely snow-free near the end of summer. Meltwater from snow, Rotch Dome and the three small glaciers, and a high annual precipitation rate results in the presence of considerable surface water during the summer months. Flat lying areas of the Peninsula favour the retention of this surface water giving rise to temporary waterlogged areas, lakes and pools. Once these systems have become saturated, the excess surface water flows through a multichannel drainage basin (López-Martínez et al. 1996).

A seasonal monitoring was conducted over a microbial community during the austral summer of 2006-07 at Byers Peninsula (Livingston Island, South Shetland Islands, 62°34'35"S - 61°13'07"W). At a glance, this community is purple coloured with a brittle and non uniform surface following the microtopography of the gravel underneath. Its matrix was formed by narrow cyanobacterial trichomes (<1 μm) of the genus *Leptolyngbya*. This mat had a striking diversity of cyanobacteria, with at least 9 additional cyanobacterial filamentous taxa of variable diameter. Diatoms were especially scarce in this mat but a surprising diversity of other organisms was found, belonging to 3 supposed trophic levels, primary producer, consumers and detritivorous. These sorts of assemblages are widespread along the depressed areas of the central plateau of Byers Peninsula as the main biological feature of the water network of lakes and ponds (Toro et al. 2007). The oligotrophic inland ponds and lakes of the Byers Peninsula often present thick microbial mats similar to those found in other maritime Antarctic freshwater ecosystems such as

those from King George Island (Vinocur and Unrein 2000) and Signy Island (Heywood 1967).

Experimental setting and analytical methods

The surveyed community was sampled at different moments (from November 11th of 2006 to February 10th of 2007) by metal cores (15 mm inner diameter), trying to avoid sampling in the proximities of previously sampled area. Three photosynthesis vs. Irradiance (PvsI) curves were carried out to describe the photosynthetic patterns and acclimation of the community along the summer season. Two of PvsI curves were done in spring conditions, comparing snow covered condition with early uncovered patches, and the third one was assayed at mid January completely ice and snow free. Besides, through the season, C and N uptake rates and N_2 fixation were measured in parallel. Simultaneously, mat samples for biomass estimation, particulate organic carbon (POC) and taxonomic determinations were sampled. Samples for biomass estimation and taxonomic surveys were immediately frozen and stored for shipping to our laboratory in Spain. The experiments which required *in situ* incubation (C and N uptake rates) were carried out in triplicate and, in order to maintain conditions as natural as possible, all the incubations were made *in situ* with surrounding water and using the appropriate tracer for estimating the uptake rates (Na $H^{13}CO_3$ (98% ^{13}C.Isotec), $(^{15}NH_4)_2SO_4$ (98% ^{15}N, CIL. Andover, MA) and $K^{15}NO_3$ (99.9% ^{15}N, Isotec. Miamisburg, OH)) were added at an approximate concentration of 10% of the natural concentration of the light isotope to avoid methodological artefacts of excessively high concentrations. Those incubations were carried out at the sampling site in Whirl-pak® bags (60 ml. Nasco) for two hours, under natural sun exposure and using light neutral filters for PvsI curves ,In the case of C uptake ratios and PvsI curves the two-hour incubation period was stopped adding 3 ml of 1N HCl per bag. In the case of ^{15}N derived nutrients the incubation was stopped by rinsing several times with GFF-filtered pond water, Afterwards, they were stored frozen and shipped to the laboratory. More detailed protocols are described in Velázquez and co-workers (2011). Water samples for chemical

analyses of dissolved compounds were filtered through pre-combusted Whatman GF/F filters within 2 h of collection. The samples were frozen immediately and stored at -20° C until analysis was conducted. Inorganic dissolved nutrients were quantified following APHA Standard Methods (1992). The transmitted light was measured by 2π quantum sensor, Li-192, which was connected to a datalogger Licor LI-1000. In laboratory, samples were weighed and dried out at 60°C during 24 hours. After grinding, isotopic enrichment was determined by IRMS Micromass-Isochrom mass spectrometer. Dissolved inorganic carbon (DIC) was calculated from water alkalinity, which was measured after tritation with HCl using a pH shift indicator (phenolphthalein) of equivalence end point pH. But Dissolved inorganic nitrogen (DIN) values were estimated from previous experiments.

Physical parameters

To describe the light conditions under the snow, irradiance data were collected from a snow and ice profile accumulated through all the winter in the watershed beside the study site. The transmitted light throughout the snow was measured by a 2π quantum sensor (Li-Cor, LI-192, which was connected to a datalogger (Li- Cor LI-1000). For light profiles, data were fitted to the following single exponential decay equation:

$$Y = ae^{-bx} \qquad (1)$$

Where, a is a constant given by the physical environment which means the surface scattered light on the snow; b is the light extinction coefficient of the snow profile and the expression e^{-bx} marks the percentage of the incident light at the cover snow surface reaching a given depth. Light and air temperature measurements during the whole season were collected by an automatic weather station (Campbell Scientific Co.) placed close to sampling site.

Automatic temperature dataloggers (Tidbit. Onset Co.) were placed on the community throughout the whole season, registering at 36 minutes intervals. In those

representations, raw data were smoothed by a LOESS algorithm, using 3 polynomial degrees in order to stress the temperature and light trends.

Biomass estimation

Metazoan biomass fractions of community were counted at every sampling event. Cores of 5 mm of inner diameter were gently disaggregated and mounted on microscope slides. Microscope photographs were taken in order to measure the parameters to assess the biovolume of every taxonomical group. Biovolume and carbon content were estimated using Benke and co-workers (1999), Burgherr and Meyer (1997) and Meyer (1989) for tardigrades, nematodes and rotifers. However, the ciliates biovolume was calculated by measuring cell dimensions and assuming simple ovoid forms and a correspondence of 0.19 pgC μm^{-3} (Putt and Stoecker 1989).

Total hyphal length and fungal biomass were also estimated by fluorescence microscopy. Mats were cored in 5 mm in diameter samples. Those were disaggregated and re-suspended in distilled water. Calcofluor white stain (Fluka) was used because it binds to chitin and cellulose in cell walls. After 5 minutes, samples were washed with KOH 5 % (v/v) final concentration 0.05 ml of re-suspended biomass were mounted in a Neubauer chamber slide (Brand GmbH) and excited by blue light (Olympus Inc. G2A. EX: 510-560, DM: 575, BA: 590). Up to100 microscopic fields were counted for hyphal length estimation according to Newman (1966) and biovolume was estimated assuming a hyphal cylindrical mean of 1.5 µm through the total hyphal length. Carbon content of the fungal section of the microbial mats was calculated according to Bloem and co-workers (1995).

Trophic web

To assess the possible trophic relationships within the community features, microbial mat samples were disaggregated in a petri dish and the different compartments of the community were sorted helped by a stereozoom microscope (Leica MZ75). After classification process, samples were dried at 65.5°C in a desiccation stove for about 48

hours. Then the natural abundances of ^{13}C were analyzed by triplicate in a mass spectrometer (IRMS Micromass-Isochrom) with the aim of displaying the trophic connection between the organisms. In the same way, natural abundances of ^{15}N into the community were also surveyed to assess the different trophic levels of the ecosystem (Post 2002). Dissolved organic matter ^{13}C signal were determined by quadruplicate as follows. A piece of mat was pressed gently against a nytal 30 µm sieve pore size diameter, the fraction <30 µm was centrifuged (5 min, 10000 rpm), the supernatant was again filtered by a 0.22 µm hydrophilic membrane and concentrated by evaporation at 40 ° C under vacuum. Then it was analyzed by mass spectrometry (IRMS Micromass-Isochrom).

Nitrogen uptake rates

Nitrogen species uptakes were measured by means of stable isotopes techniques and acetylene reduction activity. The rates of N_2 fixation (Nitrogenase activity) were determined as the acetylene reduction activity (ARA) as described by Fernández-Valiente, et al. (2001). Nitrogenase assays were made at the same location and very low activity was found along all the sampling season and collected data were close to our detection limits, so no results are presented here.

Nitrate, ammonium and urea uptake rates were measured using urea-^{15}N, $(^{15}NH_4)_2SO_4$ (both 98% ^{15}N, CIL. Andover, MA) and $K^{15}NO_3$ (99.9% ^{15}N, Isotec) from a stock solution of 30, 2 and 4 µgN ml^{-1}, respectively, for incubations in each assay. The appropriate tracer was added to Whirl-pak bags at an approximated concentration of 10% of the natural concentration of the light isotope, as measured the previous years in the same area. The urea was not measured in the environment and was added at the same concentration than dissolved inorganic nitrogen (DIN).

Mathematical analysis

Photosynthesis and irradiance data were fitted to Platt photosynthesis model (Platt et al. 1980), which follows a hyperbolic tangent equation at each temperature, using Sigmaplot

software (Systat Software Inc.). Here, Ps is defined as estimated maximum photosynthetic rate, α as the initial slope of the curve that means photosystems efficiency and E as the irradiance in each interval of the curve.

POC data were fitted to a polynomial quadratic equation with under a normality test of Shapiro-wilk (W) of 0.935. Here the intersection with the vertical axis means the initial state of the community on terms of particulate carbon content at the beginning of the season and how it is depleted by respiration and lixiviation throughout the season. Carbon community assimilation rate (CCAr) was fitted to a log-normal distribution. It was elected due to the mean values were low, variances large, absence of negative values and the skewed shape of the plotted data.

$$f(x) = \frac{1}{x\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2\sigma^2}(\log(x) - \mu)^2\right) \qquad (2)$$

Where appropriate, t-test or ANOVA with Tukey post-hoc testing were used to compare replicated measurements. SPSS Statistics 17.0 (SPSS Inc., 2008) and Sigmaplot 11.0 (Systat Software Inc.) were used for all statistical and procedures.

Ecological network analysis

In order to describe the emergent properties of the calculated simulations, we applied several indices from Ecological Network Analysis. Ecological Network Analysis (ENA) was defined by Fath and co-workers (2007) as a "*system-oriented methodology to analyse within system interactions used to identify holistic properties that are otherwise not evident from the direct observations*". Indices were computed using the program Ms Excel 2007, following the methodology described by R. Ulanowicz (Ulanowicz 2004; Allesina and Ulanowicz 2004). The total activity of the system, described by the sum of all the flows, is called the **total system throughput** (*TST*). The activity of each compartment, called the throughput, is the sum of all flows entering that compartment. To characterize the geometry of the flows, topological indices are calculated. The **ascendency (*A*)** (written with the spelling defined by Ulanowicz (1986)) is the product of the *TST* and an

information index corresponding to the **Shannon index of the flows** (*H*). This second term of the product is called the **Average Mutual Information** (*AMI*). This term will be the highest when the information about where to go next is high for an atom of carbon leaving one compartment, i.e. the trophic pathways are highly specialised. Alternatively, this term will be low if the carbon has low information, with many possible pathways of equal magnitude; such a system is considered redundant, and ascendency will be low. The ascendency is more informative on the organization of the system when it is expressed as a percentage of the **development capacity** (*DC*), which is the maximum potential ascendency (when specialisation is maximised).

The ratio of ascendency to development capacity can be defined at the total system level (relative ascendency) or at the level of the internal food web, where flows to the outside are disregarded (internal relative ascendency). The development capacity is the sum of ascendency, redundancy and information related to external exchanges. The internal development capacity is the sum of internal ascendency and internal redundancy. Here, the **redundancy** (Red) is tied to the effective multiplicity number of parallel flows by which medium passes between any two arbitrary system components. Also, the **Finn Cycling Index** (*FCI*; (Finn 1976)) was calculated as the ratio of the sum of carbon flows in cyclic pathways to the sum of all carbon flows in the systems. The ratio Detritivory/herbivory was calculated as the sum of flows of consumption of non-living material divided by the sum of flows of ingestion of autotrophic organisms (Ulanowicz 1986; Ulanowicz 1997). Below those terms are developed, presenting the equations and where they come.

Here 5 different scenarios (scenario 2-6) are presented related to the estimated field data. In the scenario 2 the primary production has been raised up a 35 %, contrastingly to the scenario 3, where a 35% of the primary production has been reduced. Also, at scenarios 4 and 5 the predation pressure has been raised up or reduced 35% of

the basal model regarding to the features of ciliates, nematodes, tardigrades and rotifers. For the scenario 6 an increase of 70% of the POC in the community has been applied.

(a) Model development

To explore the different roles of primary producers, metazoan and the POC budget in the community, the functional groups were distributed in 13 compartments (called nodes in the network analysis). Those were Algae (*alg*), Cyanobacteria (*cyan*), Diatoms (*diat*), Bacteria (*bact*), fungi (*fung*), Nematodes (*nem*), tardigrades (*tard*), Rotifers (*rot*), Dissolved Organic Carbon in the community (*DOC*), Particulate Organic Carbon in the community (*POC*), C inputs (*imp*) and respiration and other C losses (*exp*). To represent the compartments as a matrix, Pajek software was used (Batagelj and Mrvar 2006) and some assumptions were taken:

- The role of fungi was comprised in one compartment, instead of knowing the different live cycle stages they pass through, as zoospore formation and so on.

- Virus compartment was not included because the carbon budget they represent is considered to be minimum compared with other compartments of the system, but their influence is enclosed as different flows to POC and DOC compartments.

- Thresholds for some biological and physical processes were applied to constrain the calculated flow to ranges that resulted in realistic values for planktonic processes (Niquil et al. 2006; Niquil et al. 2011).

- The different scenarios were modified ±35% of some field values to mark the differences between them.

Averaged values were taken to develop this steady-state matrix model and the real aim was to evaluate the trophic network topology.

(b) Sensitivity analysis

A sensitivity analysis was performed to assess the dependence of the calculated terms on variations in the values of the field values (table 2). Mass balance calculations from field assays were made over ciliates, fungi, nematodes, tardigrade, rotifers and POC. Those 5 field data values were varied by ±10% and ±50% one at a time and a new set of flows calculated for this perturbed situation. Results for each flow were expressed as a sensitivity index SI (e.g.(Niquil et al. 2006; Richardson et al. 2004) over the ENA terms:

$$SI\ for\ ith\ term = \frac{\left(\frac{\left|value_{i,p} - value_{i,o}\right|}{value_{i,o}}\right)}{Modified\ percentage} \cdot 100 \qquad (3)$$

Where $value_{i,p}$ and $value_{i,o}$ are the values for the *ith* term in the perturbed and original situations. The values indicate that the 10% or 50% variation of the field value leads to a 10% or 50% variation in the considered flow value.

(c) Network equations

The performance of the system as a whole at processing material and energy can be quantified using information theory. In particular, the complexity of process interactions can be parsed into separate terms that distinguish organized, efficient performance from the capacity for further development and recovery from disturbance.

The whole –system status address was initiated by MacArthur (1955), who applied **Shannon´s information measure** (H) to the flows in an ecosystem network as,

$$H = -k \sum_{i,j} \left(\frac{Tij}{T_{\cdot}}\right) \log \left(\frac{Tij}{T_{\cdot\cdot}}\right) \qquad (4)$$

Where H is the diversity of flows in the network, k is a scalar constant as $k=T_{\cdot\cdot}=1$ (the total system throughput) in normalized matrices networks flows, and $T_{\cdot\cdot}$ signifies the sum of T_{ij} over all combinations i and j. Then (Rutledge et al. 1976) employed a more

recent notion of conditional probability to decompose MacArthur´s index into two complementary terms. If (*Tij/T··*) is the unconditional probability that a flow from *i* to *j* occurs, then (*Tij/T.j*) is the conditional probability that the quantum of flow proceeds to compartment *j*, given that it had issued from component *i*. That is, *H* can be decomposed as

$$H = \mathrm{AMI} + H_c \qquad (5)$$

Where

$$AMI = k\sum_{i,j}\left(\frac{Tij}{T_{..}}\right)\log\left(\frac{TijT_{..}}{Ti.T.j}\right) \qquad (6)$$

and

$$Hc = -k\sum_{i,j}\left(\frac{Tij}{T_{..}}\right)\log\left(\frac{Tij^2}{Ti.T.j}\right) \qquad (7)$$

AMI is called the ***average mutual information*** inherent in the flow structure; *Hc* is the residual (conditional) diversity/freedom. In other words, the overall complexity of the flow structure, as measured by MacArthur´s index, can be resolved into a component that gauges how orderly and coherently the flows are connected and a residual that measures the disorder and freedom that remains. (Rutledge et al. 1976) focused upon *Hc* as a more appropriate measure of ecosystem maturity (in the sense of Odum (1969)) than MacArthur´s ambiguous index. Ulanowicz (1980) suggested that *AMI*, instead of *Hc*, is more indicative of the web development status of an ecosystem, because *AMI* measures the average amount of constraint exerted upon an arbitrary quantum of currency in passing form any one compartment to the next ((Ulanowicz 1997; Latham and Scully 2002). *AMI*, however, has no physical dimensions. That is, when *T··=k* in the equation (4), it is called the system network ascendency, *A*. Ascendancy thus combines the total activity, or power of the system (*T··*), with the organization by which the component processes are linked (*AMI*) (Latham and Scully 2002). It gauges how well the system is performing at processing the given medium. Initially, it had been thought that an ecosystem would develope so as to maximize its ascendency (Ulanowicz 1980), but the disparity nature of

such a statement eventually overlay mechanical and deterministic (Mueller and Leupelt 1998). So, it is more appropriate to speak of a propensity for ecosystems to increase in *A* (Ulanowicz 1997). Beside, *Hc* can be scaled by *T··* to yield what is called the system **overhead,** *Φ* (Ulanowicz and Norden 1990), as

$$\Phi = -\sum_{i,j} Tij \log\left(\frac{Tij^2}{Ti.T.j}\right) \qquad (8)$$

And *H* itself can also be scaled to produce what is termed the ***system´s developmental capacity***, *DC*,

$$C = -\sum_{i,j} Tij \log\left(\frac{Tij}{T..}\right) \qquad (9)$$

Accordingly, relationship (4), when scaled becomes

$$C = A + \Phi \qquad (10)$$

The decomposition of this implies that increasing ascendency usually arises at the expense of the complementary overhead, *Φ*. Difficulties with simulation aside, knowing the relative values of *A* and *Φ* can nonetheless indicate the status of the ecosystem as link-density, average flow into or out of a typical node, and so on. Ulanowicz and Wolff (1991) demonstrated that taking the base of the logarithms to the power (*Hc/2*), yields a convenient measure of the effective link-density. Zorach and Ulanowicz (2003) show the deeper connection between *Hc* and link-density, which is raising the base of the algorithm to the power (*Hc/2*) yields precisely the weighted geometric mean of the link density that would be calculated using conventional algebra. Arguing from dimensional considerations, it can be inferred how raising the logarithmic base to the power *AMI* should correspondingly estimate the number of **trophic "roles"**, *R*, in the network, that is, it corresponds roughly to the effective number of trophic levels in the network. That is,

$$R = N^2 \cdot F = \frac{N}{Conn} = \frac{F}{Conn^2} = \prod_{i,j}\left(\frac{TijT..}{Ti.T.j}\right)^{\left(\frac{Tij}{T}..\right)} \qquad (11)$$

Where, in a weighted network, *N* is the **effective number of nodes** participating in the network and *F* is the **effective number of flows**. Solving those equations, *AMI* can be

estimated as log *R (Ulanowicz 2004).* The Finn Cycling Index (Finn 1976) utilizes the Leontief matrix to assess the amount of material cycling within the ecosystem. The formula, derived from the inverse Leontief matrix is (Allesina and Ulanowicz 2004):

$$FCI = \sum_{i=1}^{n} \frac{S_i}{TST} \frac{l_{ii}-1}{l_{ii}} \quad (12)$$

Where l_{ii} is the *ith* coefficient along the diagonal of the Leontief matrix, and S_i is the inflow to the *ith* compartment.

So, the developed matrix model displays weighted interactions between the different nodes, that were rotifers, nematodes, ciliates, virus, fungi, green algae, diatoms, cyanobacteria, bacteria and tardigrades. Besides, the inputs and outputs were also implemented as different nodes.

RESULTS

Composition of the community

Matrix of the microbial community consisted of two thin cyanobacteria of the morphotypes I (1 μm in diameter) and J (3 μm in diameter) sensu Broady and Kibblewhite (1991) compared to the Oscillatorian diversity in Ross Island and Southern Victoria Land in continental Antarctica. Intermixed with the matrix filaments there were also many unicellular cyanobacteria (1.5 μm in diameter). Thicker cyanobacteria from morphotype C (5.5-6 μm in diameter) were also present within the mat. According to (Anagnostidis and Komarek 1988) all these morphotypes could be assigned to different species of genus *Phormidium* (Broady and Kibblewhite 1991). Other filamentous cyanobacteria were also present in the surface layer, such as different *Phormidium* species assigned to morphotypes B and K (4-5 μm in diameter) and morphotype E (11 μm in diameter), which were also abundant. Besides, an unidentified filamentous cyanobacterium with a dark thick sheath (18 μm in diameter) was observed abundantly at the mat surface layer. Cyanobacterial cells of 5.5 μm in diameter were observed too, emerging from broken sheaths; those probably belong to the family Phormidiaceae because of its thick, lamellated and coloured sheath. Diatoms were also present but at low density. Abundant microcolonies of Nostoc sp. appeared in the deepest layer.

In early November, Chlorophyte genera were one of the dominant features under microscopic observations but their abundance decreased with the seson until becoming marginal, or appearing as resistance forms at the end of the season. No differences were found attending to matrix components, but a slight increase in the larger cyanobacteria genus, i.e. *Phormidium* 5 μm in diameter. A *Tolypothrix* species was found also, but its presence was absolutely marginal.

Regarding to metazoan composition of the community, species resolution is out of the scope of this paper. However, the bacterial feeders nematodes *Geomonhystera* spp., *Plectus* spp., *Teratocephalus* spp. and fungal feeders nematodes *Aphelenchoides* spp. have

been described in very similar ecosystems in Byers Peninsula (Nielsen et al. 2011). About ciliates, genera described by Petz (2003) for the area were *Heterotrichea* spp., *Hypotrichia* spp., *Oligotrichia* spp., *Phyllopharyngia* spp., *Suctoria* spp., *Nassophorea* spp., *Colpodea* spp., *Prostomatea* spp., *Peniculia* spp., *Scuticociliatia* spp., *Hymenostomatya* spp. and as principal genera *Stichitrichia* spp., *Litostomatea* spp. and *Peritrichia* spp.. Those were distributed as follow: 36% bacterivores, 9% cyanobacterivores, 23.3% algivores, 29% predators, 1.3% fungivores and 2% omnivores. Regarding to Tardigrada genera, *Macrobiotus* spp., *Minibiotus* spp., *Aucuntuncus* spp. and *Dactilobiotus* spp., were identified. Those identifications were made comparing eggs´ microphotographs from sediment cores of Limnopolar Lake, 300 m beside the studied community. The feeding habits of those genera are mainly alguivores, detritivores and fungivores, but there are some descriptions that they predate on rotifers also (Sohlenius and Boström 2006). Rotifers were not identified, although they likely belong to *Bdelloidea* and *Monogononta* classes.

The percentages of bacterial families described in the community are 50% α-proteobacterias, 6,68% β-proteobacterias, 0.42% Firmicutes and a 43,2% of unknown items (Salinero 2007). Finally the virus population over the community appear to be cyanophages and other eukaryotic virus presumably ssDNA (A. Lopez-Bueno Pers. Comm.)

Light and temperature profiles

As the community was covered by snow and ice at the beginning of the season, light profiles indicated that the light reached 39 cm depth, below this depth the light was very close to zero. It was calculated by a light extinction coefficient of 11.9 after fitting the data to a single exponential decay of two parameters (Stibal et al. 2007). At the first sampling event, only the 2% of the incident irradiance reached the community. Then, due to snow melting, it grew to 13% at next sampling event until the whole amount of snow was depleted by melting and evaporation at early December. Afterwards, the community kept uncovered of snow and ice until the end of the season (figure 1).

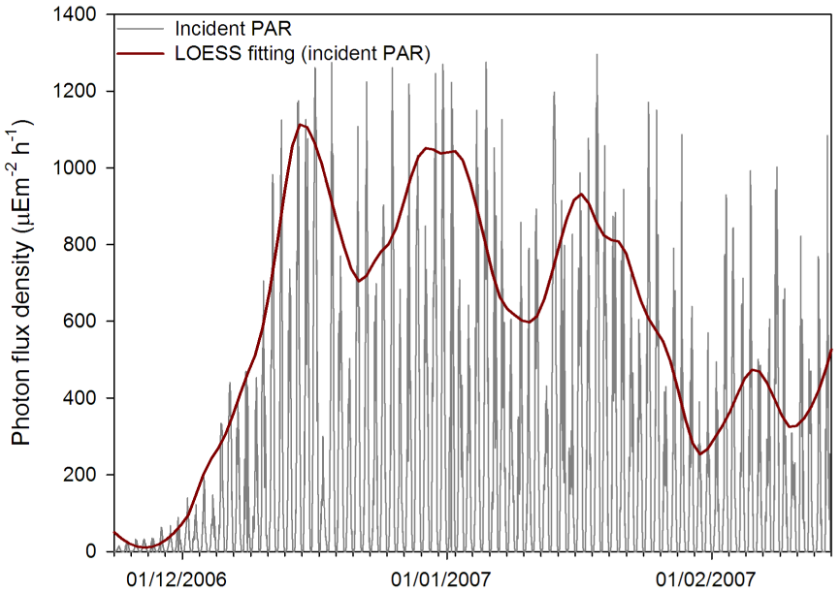

Figure 1. Dynamic of incident Photosynthetic Active Radiation at the surface of SW Pond (Byers Peninsula, South Shetland Islands, Antarctica) during summer season of 2006-07. Values are corrected by extinction coefficient of the snow cover, which remained until the 1st week of December 2006.

The community shows a daily average temperature range of 9.52±4.8 °C (figure 2), but the maximum temperature reached up to 18.96 °C, while in the same day, by night temperature was -0.07 °C, that means a range maximum of 19.03 °C. Consistently, temperature within the microbial mat surface was higher than air records. This difference after the LOESS fitting was, on average, ca. 3.5° C.

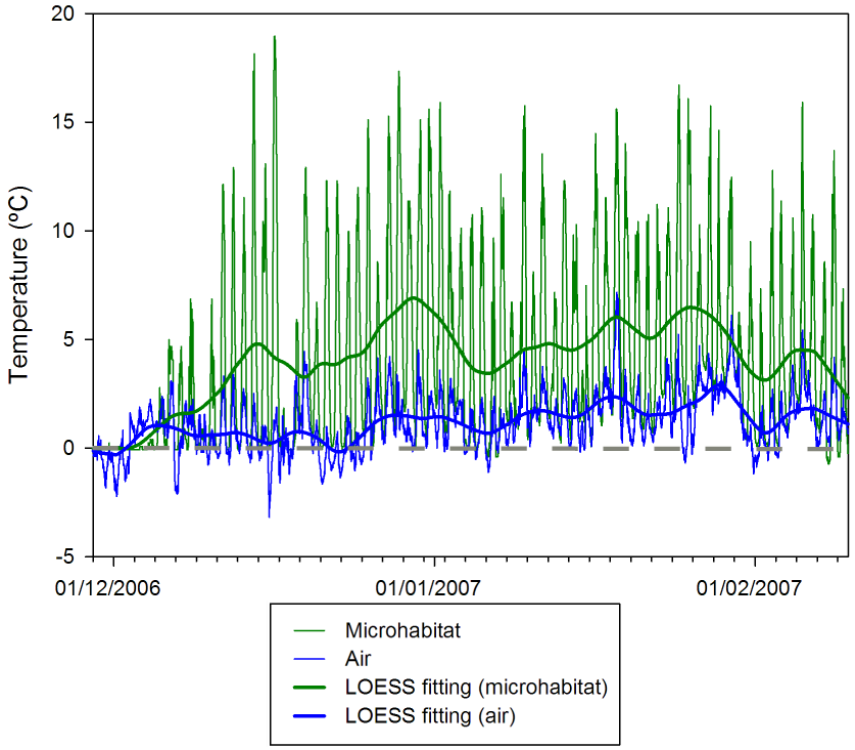

Figure 2. Temperature summer regime at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica).

Nutrients dynamics

Analysis of nutrients in the overlaying water indicated that this pond can be considered an oligothrophic environment with values of phosphorus between 0.04 -0.29 µM. with N:P molar ratios ($N\text{-}NO_2^-$+$N\text{-}NO_3^-$+ $N\text{-}NH_4^-$)=DIN and $P\text{-}PO_4^{3-}$)of 2.95±1.7 at the beginning of the season that is compensated reaching values of 13.5±1.9 at midsummer due to metabolic dynamic processes. N_2 fixation activity was scarce, and probably our results only reflect values close to the baseline, with a slight decrease activity trend from early to mid summer (data not shown). Moreover, C/N values along the season were 10.8±1.3, almost twofold of standards bare-field ratios of ecosystem equilibrium.

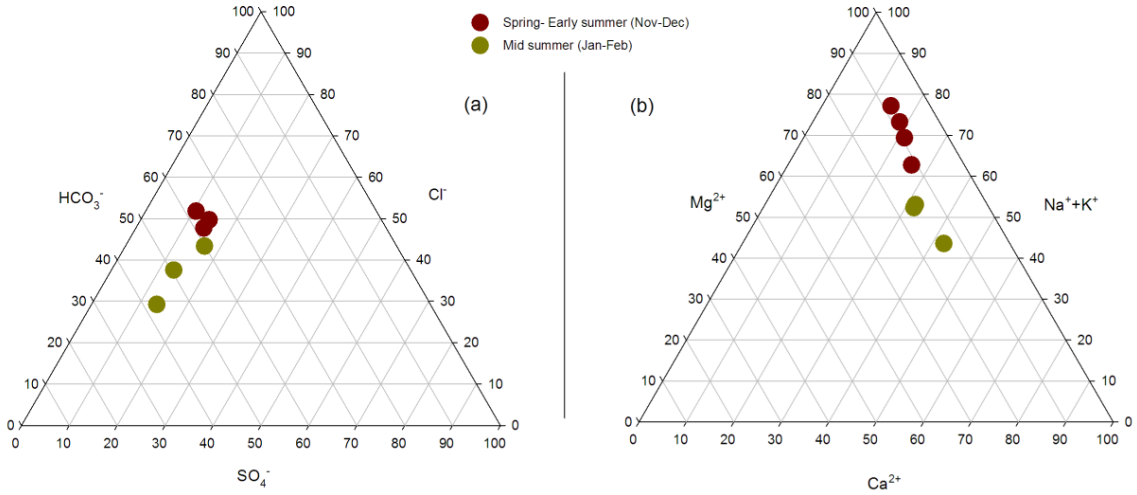

Figure 3. Ternary diagrams of main anions concentration (a) and main cations (b) of the overlaying water of SW pond microbial mat.

Water nutrient analysis regarding to dissolved inorganic nitrogen (DIN) show the dominance of NOx species of the total nitrogen of the community surrounding water. Ternary diagrams of main ions show a calcium carbonate influence of the lithology and in spite of the sampling area being close to the sea, oceanic influence decreased across the summer season (figure 3). Deuterium samples of the overlaying and running water of the community (δD=-67.3 ± 2.8) and the absence of bulk snow banks upstream indicated that wetland water source comes from permafrost's active layer that melts every summer. This permafrost origin and the liquid precipitations support the community hydric balance

every year. However, this assumption is confirmed by the increase of Ca^{+2} and the decrease of monovalent ions (Na^{+}-K^{+}) in the surrounding waters.

PvsI curves

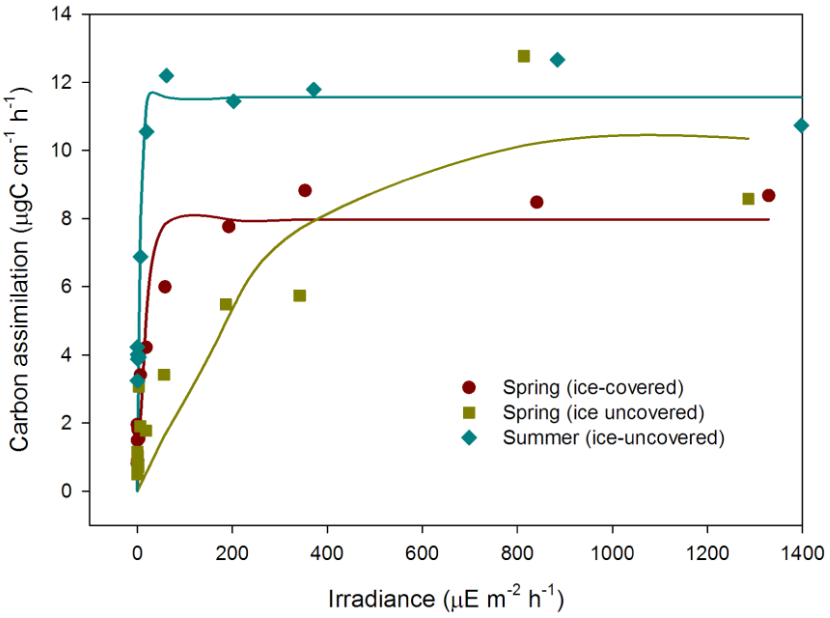

Figure 4. Photosynthesis *versus* irradiance curves of the community. Lines show photosynthetic versus irradiance fitted values to Platt and co-workers (1980) model. Squares, circles and diamonds are the average of three values at every irradiance.

The fitted PvsI curves (figure 4) work as good descriptors of the community autotrophic compartment. The parameters estimated by different models of photosynthesis vs. irradiance (table 1) exhibit similar behaviors, but the fitting of the Platt models to the obtained data was robust, with Pearson correlation coefficients (R^2) between 0.94 and 0.97. The first slope of the curves of spring (ice covered) and summer (ice uncovered) are almost vertical, with α values of 0.32±0.10 and 1.41±0.45 (µg C $cm^{-2}h^{-1}$ (µE m^{-2} $s^{-1})^{-1}$). But the transition period of spring (ice uncovered) outline a photosynthesis efficiency lower 0.03±0.01 (µg C $cm^{-2}h^{-1}$ (µE m^{-2} $s^{-1})^{-1}$). Then, the curves saturate asymptotically showing no photoinhibition under maximum natural irradiances. Maximum photosynthesis (P_m) of the ice uncovered stages was in the same range, both in Spring and Summer, 10.36±1.36 µgC m^{-2} h^{-1} and 11.56±0.87 µgC m^{-2} h^{-1} respectively and higher than the community ice covered (7.98±0.55 µgC m^{-2} h^{-1}). Besides, E_k values reflect different community conditions and E_k of summer uncovered situation (357.7 µE $m^{-2}s^{-1}$) is probably reflecting a

combination between efficiency of both photosynthetic apparatus of Chlorophyte and Cyanobacteria.

Table 1. Photosynthesis versus irradiance curve parameters fitted to different models.

Communities and models	P_{max} ($\mu g\ C\ cm^{-2}\ h^{-1}$)	P_{max} ±SE	α ($\mu g\ C\ cm^{-2} h^{-1}\ (\mu E\ m^{-2}\ s^{-1})^{-1}$)	α ±SE	Pearson R^2 adjustment	Light saturation index (E_k) ($\mu E\ m^{-2} s^{-1}$)
Exponential-Saturation Fit (Webb model)						
Spring- ice covered	8.10	0.52	0.39	0.12	0.97	20.84
Spring- ice uncovered	10.30	1.42	0.04	0.02	0.93	245.54
Summer- ice uncovered	11.60	0.85	1.88	0.64	0.96	6.16
Waiting-in-line Curve						
Spring- ice covered	11.11	2.52	0.05	0.02	0.94	206.78
Spring- ice uncovered	10.73	2.24	0.04	0.01	0.95	302.81
Summer- ice uncovered	16.38	5.72	0.09	0.04	0.82	186.27
Michaelis-Menten Curve						
Spring- ice covered	8.52	0.45	0.62	0.19	0.98	13.69
Spring- ice uncovered	11.48	0.63	0.07	0.03	0.93	167.74
Summer- ice uncovered	11.87	1.24	3.33	1.56	0.94	3.56
Hyperbolic Tangent Curve (Platt Model)						
Spring- ice covered	7.98	0.55	0.32	0.10	0.97	24.65
Spring- ice uncovered	10.36	1.36	0.03	0.01	0.94	357.67
Summer- ice uncovered	11.56	0.87	1.41	0.45	0.96	8.22

Carbon dynamics

Carbon photoassimilation rate along the season followed a log-normal distribution (R^2=0.82) (figure 5). The vertical line in the figure shows when it is assumed that the bulk activity of the community is over because temperatures dropped below zero. The POC values, with some variations, remained between 3000 to 5000 µgC cm^{-2}. Therefore, this fitting could be a mathematical artefact form the model fitting and the POC values just outline a straight line, marking non large variations through the growing season. So, those results should be interpreted cautiously.

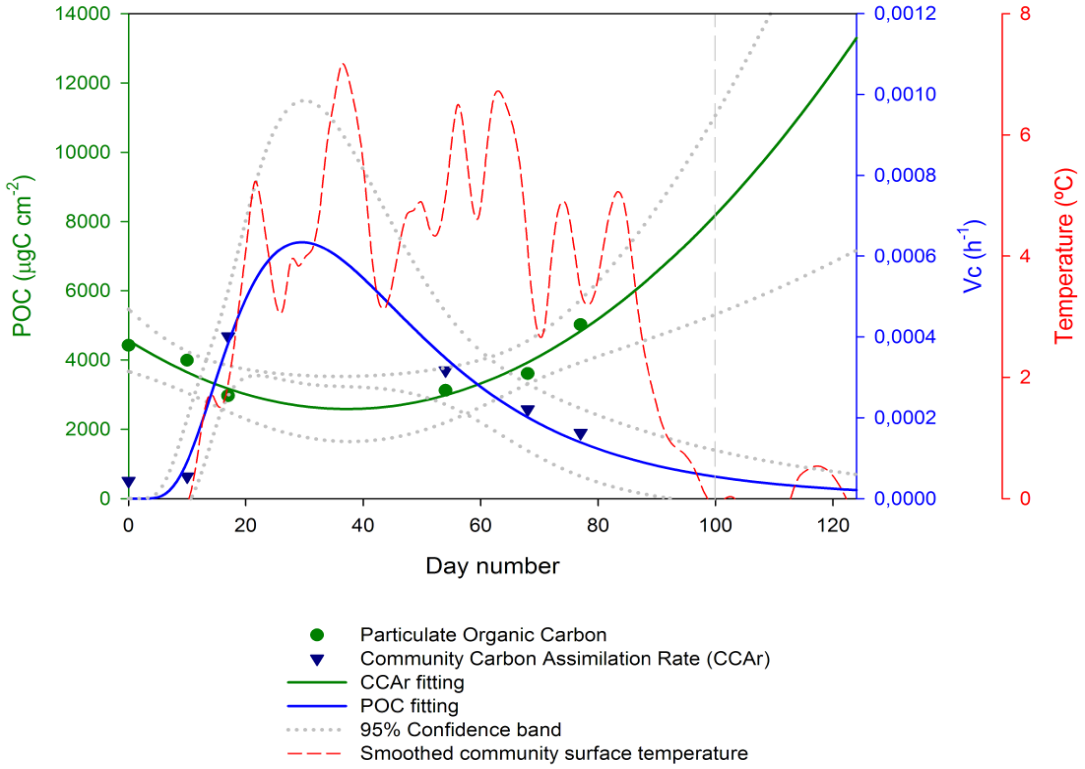

Figure 5. Carbon dynamic of SW Pond benthic microbial community (Byers Peninsula, South Shetland Islands, Antarctica). Community Carbon assimilation Rate (CCAr) values were fitted to a log-normal distribution with a R^2=0.91. Particulate Organic carbon (POC) values were fitted to a polynomial quadratic equation with a R^2=0.82. Vertical dashed line marks the estimated end of the season, when temperature drops to near 0 °C values.

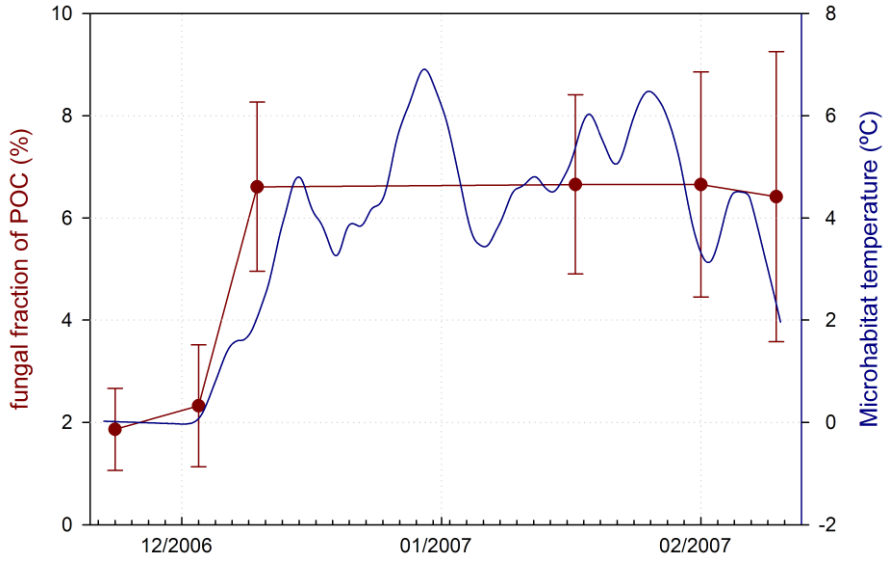

Figure 6. Fungal fraction of the POC estimated during summer season of 2006-07 at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica).

Regarding to community's heterotrophic metabolism the, hyphal biomass followed the rising of the temperature pattern (figure 6), reaching a plateau stage when

temperature remains over the freezing point (figure 2). The fungal biomass increment is prompted by raising temperature records of previous days. Attending to the metazoan quantification of population density, it is clear that the predation pressure is lead by the phylum Nematode; in fact this group represented the maximum organic C amongst metazoans, at least from mid-summer (figure 7).

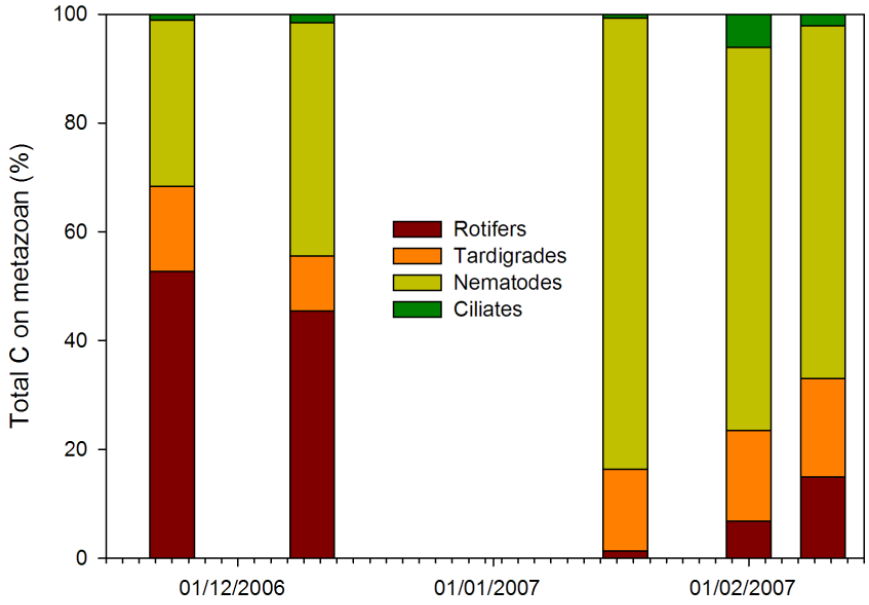

Figure 7. Total carbon percentage estimated on metazoans during summer season of 2006-07 at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica).

Nitrogen dynamic

The ammonium and nitrate uptakes follow the same pattern during almost the whole season (figure 8). But at the beginning, ammonium cope the DIN concentration values which is followed by a raising tendency in ammonium uptake (figure 8a), contrary to nitrate. Then, nitrate drives DIN concentration (figure 8b) and the tendency switches between both compounds. Thereafter, the uptake of nitrate seems to be favoured, taking higher δ values for the whole season.

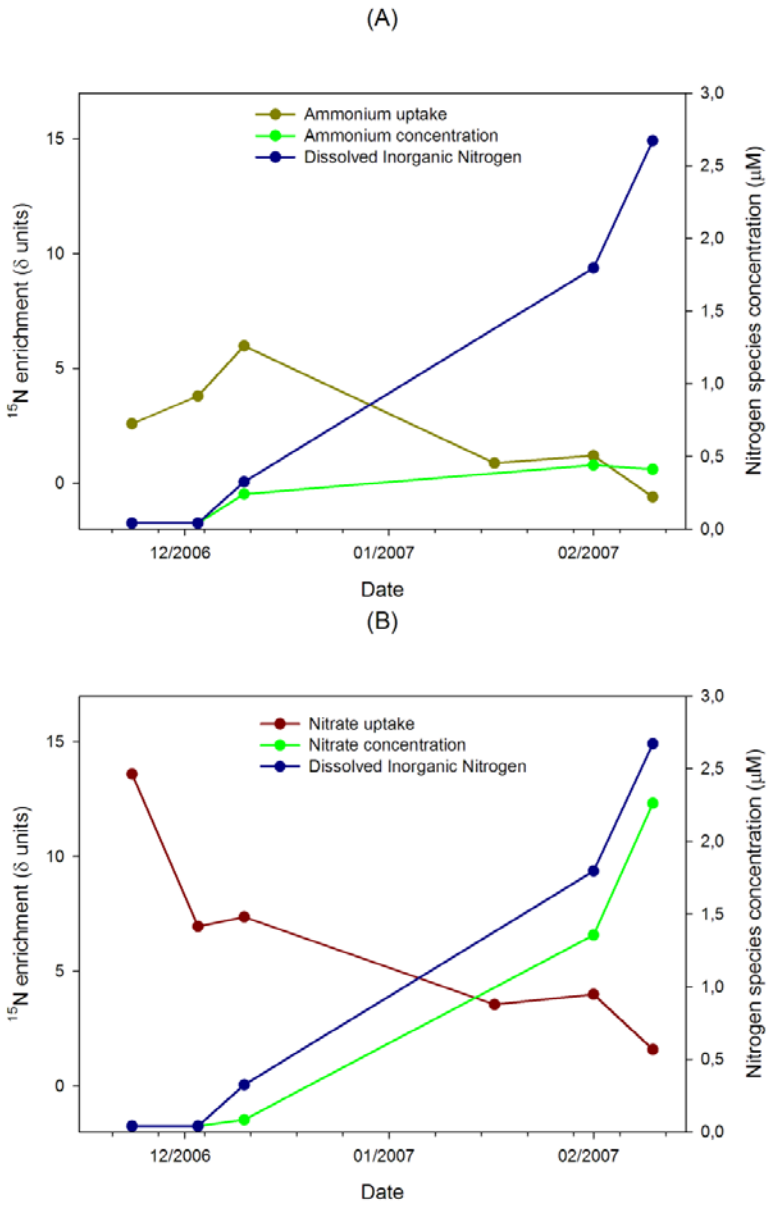

Figure 8. Ammonium (A) and nitrate (B) dynamic during summer season of 2006-07 at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica). Those values of enrichment are compared to nitrogen species concentration.

Trophic web

The isotopic profile of the community (figure 9) indicates that neither the rotifers nor the tardigrades or probably nematodes fed on cyanobacteria or diatoms but are able to feed on fungi and DOC. The partial analysis of the trophic status of the community leaves out important features of the community as ciliates and virus due to the impossibility to separate them. On the other side, isotopic signals of diatoms and mosses found within the community suggest not interfering with the other features, but diatoms are not consumed

by predators (nematodes, tardigrades and rotifers) due to their frustules. Non represented groups as ciliates should be involved in the trophic web at several levels as primary consumers and detritivorous (Petz 2003).

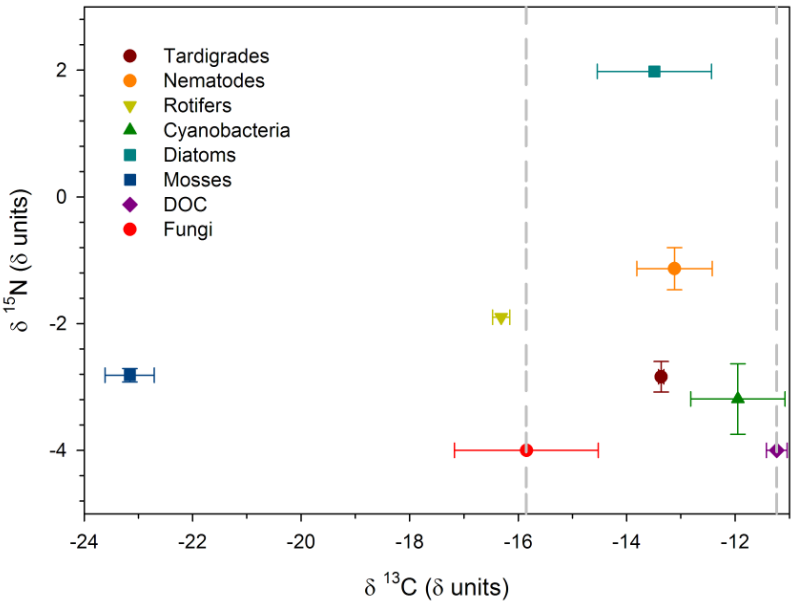

Figure 9. δ^{13} C and δ^{15} N signal of diverse features of the microbial mat community from SW Pond (Byers Peninsula, South Shetland Islands, Antarctica). Dotted vertical lines mark absence of data for the compartments fungi and DOC.

Network analysis

The carbon flow values inferred from the model are given in table 3. The total gross primary production ranged from 225 (scenario 2) to 468 (scenario3) mgC m^{-2} d^{-1} and the secondary production from 205 (scenario 4) to 422 (scenario 5) mgC m^{-2} d^{-1} (table 2). In all the proposed scenarios, but scenario 6, a value of 431 mgC m^{-2} d^{-1} for net C transformed to POC is kept (table 2). As far as individual flows are concerned to calculate the sensitivity index from the field values variations give the ecological network parameters greater sensitivity to greater modifications. Here, the most impacted parameters are *TST*, *DC*, *Φ*, *A*, A_i and *Redundancy* (table 4) in both sensitivity approaches. But they do not present linear relationship to the modifications between ±10% and ±50%.

Table 2. Flows of the steady-state model in mgC m^{-2} day^{-1}.For each flow name is defined as unit (Carbon) source compartment-TO- sink compartment.

Item	Description	Flow name	Inferred value					
			Current scenario	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
	Rotifers net production	Crotnetp	83	55	55	54	112	83
	Fungi net production	Cfungnetp	2	1	1	2	2	2
	Tardigrades net production	Ctardnetp	44	29	29	29	60	44
	Nematodes net production	Cnemnetp	180	120	120	117	243	180
	Ciliates net production	Ccilnetp	4	3	3	3	5	4
	Total gross primary production	Ctotgpp	347	225	468	347	347	347
Primary prodution	Gross primary production by Algae	CgppTOalg	12	8	16	12	12	12
	Gross primary production by Diatom	CgppTOdiat	12	8	16	12	12	12
	Gross primary production by Cyanobacteria	CgppTOcyan	208	135	281	208	208	208
Algae	Respiration of Algae	CalgTOexp	3	2	5	3	3	3
	Algae detrital POC production	CalgTOpoc	1	1	1	1	1	1
	DOC excretion by Algae	CalgTOdoc	1	1	1	1	1	1
	Algae parasited by Fungi	CalgTOfung	3	3	3	3	3	4
Diatoms	Respiration of Diatoms	CdiatTOexp	3	2	5	3	3	3
	Diatoms detrital POC production	CdiatTOpoc	1	1	2	1	1	1
	DOC excretion by Diatoms	CdiatTOdoc	1	1	1	1	1	1
Cyanobacteria	Respiration of Cyanobacteria	CcyanTOexp	62	41	84	62	62	62
	Cyanobacteria detrital POC production	CcyanTOpoc	302	302	302	302	302	407
	DOC excretion by Cyanobacteria	CcyanTOdoc	10	7	14	10	10	10
	Cyanobacteria parasited by Fungi	CcyanTOfung	60	60	60	60	60	81
Ciliates	Respiration of Ciliates	CcilTOexp	3	3	3	2	4	3
	Grazing of Algae by Ciliates	CalgTOcil	3	2	4	2	4	3
	Grazing of Cyanobacteria by Ciliates	CcyanTOcil	19	12	25	12	25	19
	Grazing of Fungi by Ciliates	CfungTOcil	5	5	5	3	6	6
	Grazing of Bacteria by Ciliates	CbacTOcil	84	84	84	54	113	113
	Grazing of Rotifers by Ciliates	CrotTOcil	2	2	2	1	2	2
	Ciliates detrital POC production	CcilTOpoc	3	3	4	2	5	4
	DOC excretion by Ciliates	CcilTOdoc	11	10	12	7	15	14
Nematodes	Respitration of Nematodes	CnemTOexp	24	24	24	16	32	24
	Grazing of Fungi by Nematodes	CfunTOnem	33	33	33	22	45	45
	Grazing of Bacteria by Nematodes	CbacTOnem	205	205	205	133	276	276
	Nematodes detrital POC production	CnemTOpoc	14	14	14	9	19	19
	DOC excretion by Nematodes	CnemTOdoc	48	48	48	31	64	64
Tardigrades	Respiration of Tardigrades	CtarTOexp	6	6	6	4	8	6
	Grazing of Fungi by Tardigrades	CfunTOtar	121	121	121	78	163	163
	Grazing of Algae by Tardigrades	CalgTOtar	3	2	5	2	5	3
	Grazing of Bacteria by Tardigrades	CbacTOtar	93	93	93	61	126	126
	Tardigrades detrital POC production	CtarTOpoc	22	22	22	14	29	29
	DOC excretion by Tardigrades	CtarTOdoc	43	43	44	28	59	58
Rotifers	Respiration of Rotifers	CrotTOexp	11	11	11	7	15	11
	Grazing of Bacteria by Rotifers	CbacTOrot	58	58	58	38	79	79
	Rotifers detrital POC production	CrotTOpoc	6	6	6	4	8	8
	DOC excretion by Rotifers	CrotTOdoc	3	3	3	2	4	4
Bacteria	Respiration of Bacteria	CbacTOexp	105	105	105	105	105	141
	Grazing of DOC by Bacteria	CdocTObac	233	233	233	233	233	314
	Bacteria mortality	CbacTOdoc	28	28	28	28	28	38
Fungi	Respiration by Fungi	CfunTOexp	13	13	13	13	13	17
	Fungi detrital POC production	CfunTOpoc	302	302	302	302	302	407
POC	Non-living POC dissoltuion to DOC	CpocTOdoc	27	27	27	27	27	37
	Grazing of POC by Rotifers	CpocTOrot	43	43	43	28	58	58
	Grazing of POC by Nematodes	CpocTOnem	172	172	172	112	233	233
	Grazing of POC by Ciliates	CpocTOcil	43	43	43	28	58	58
Imports	Net import of POC (import-export)	CimpTOpoc	431	431	431	431	431	582
	Net import of DOC (import-export)	CimpTOdoc	388	388	388	388	388	524

Table 3. Linear equations used as complements for the mass balance equations.

Nº	Item	Description	Flow name	Corresponding equation	Inferred value
		Rotifers net production	Crotnetp		83
		Fungi net production	Cfungnetp		2
		Tardigrades net production	Ctardnetp		44
		Nematodes net production	Cnemnetp		180
		Ciliates net production	Ccilnetp		4
		Total gross primary production	Ctotgpp		347
1	Primary production	Gross primary production by Algae	CgppTOalg	gppx0.3	17
2		Gross primary production by Diatom	CgppTOdiat	gppx0.3	17
3		Gross primary production by Cyanobacteria	CgppTOcyan	gppx0.4	312
4	Algae	Respiration of Algae	CalgTOexp	0.3CgppToalg	5
5		Algae detrital POC production	CalgTOpoc	0,1CalgTOfung	1
6		DOC excretion by Algae	CalgTOdoc	(CalgTOexp-CgppTOalg)0.07	1
7		Algae parasited by Fungi	CalgTOfung	(CcyanTOfung x Cfungnetp)0.05	5
8	Diatoms	Respiration of Diatoms	CdiatTOexp	CgppTOdiat0.3	5
9		Diatoms detrital POC production	CdiatTOpoc	CgppTOdiat0.1	2
10		DOC excretion by Diatoms	CdiatTOdoc	(CdiatTOexp-CgppTOdiat)0.07	1
11	Cyanobacteria	Respiration of Cyanobacteria	CcyanTOexp	CgppTOcyan0.3	94
12		Cyanobacteria detrital POC production	CcyanTOpoc	CimpTOpoc0.7	302
13		DOC excretion by Cyanobacteria	CcyanTOdoc	(CcyanTOexp-CgppTOcyan)0.07	15
14		Cyanobacteria parasited by Fungi	CcyanTOfung	CcyanTOpoc0.2	60
15	Ciliates	Respiration of Ciliates	CcilTOexp	Ccilnetp0.2	4
16		Grazing of Algae by Ciliates	CalgTOcil	CgppTOalg0.23	4
17		Grazing of Cyanobacteria by Ciliates	CcyanTOcil	CgppTOcyan0.09	28
18		Grazing of Fungi by Ciliates	CfungTOcil	CdocTObac0.02	5
19		Grazing of Bacteria by Ciliates	CbacTOcil	CdocTObac0.36	84
20		Grazing of Rotifers by Ciliates	CrotTOcil	CrotTOpoc0.29	2
21		Ciliates detrital POC production	CcilTOpoc	CcilTOdoc0.3	4
22		DOC excretion by Ciliates	CcilTOdoc	(CalgTOcil+CcyanTOcil+CfungTOcil +CbacTOcil+CrotTOcil)0,1	12
23	Nematodes	Respitration of Nematodes	CnemTOexp	Cnemnetp0.2	36
24		Grazing of Fungi by Nematodes	CfunTOnem	CfunTOpoc0.11	33
25		Grazing of Bacteria by Nematodes	CbacTOnem	CdocTObac0,88	205
26		Nematodes detrital POC production	CnemTOpoc	CnemTOdoc0.3	14

27		DOC excretion by Nematodes	CnemTOdoc	(CfunTOnem+CbacTOnem)0.2	48
28	Tardigrades	Respiration of Tardigrades	CtarTOexp	Ctardnetp0.2	9
29		Grazing of Fungi by Tardigrades	CfunTOtar	CfunTOpoc0.4	121
30		Grazing of Algae by Tardigrades	CalgTOtar	CgppTOalg0,3	5
31		Grazing of Bacteria by Tardigrades	CbacTOtar	CdocTObac0.4	93
32		Tardigrades detrital POC production	CtarTOpoc	(CfunTOtar+CalgTOtar+CbacTOtar) 0.1	22
33		DOC excretion by Tardigrades	CtarTOdoc	(CfunTOtar+CalgTOtar+CbacTOtar) 0.2	44
34	Rotifers	Respiration of Rotifers	CrotTOexp	Crotnetp0.2	17
35		Grazing of Bacteria by Rotifers	CbacTOrot	CdocTObac0,25	58
36		Rotifers detrital POC production	CrotTOpoc	CbacTOrot0.1	6
37		DOC excretion by Rotifers	CrotTOdoc	CbacTOrot0.05	3
38	Bacteria	Respiration of Bacteria	CbacTOexp	CdocTObac0,45	105
39		Grazing of DOC by Bacteria	CdocTObac	CimpTOdoc0,6	233
40		Bacteria mortality	CbacTOdoc	CdocTObac0,12	28
41	Fungi	Respiration by Fungi	CfunTOexp	(CcyanTOfung+CalgTOfung)0.2	13
42		Fungi detrital POC production	CfunTOpoc	CimpTOpoc0.7	302
43	POC	Non-living POC dissoltuion to DOC	CpocTOdoc	CimpTOdoc0.07	27
44		Grazing of POC by Rotifers	CpocTOrot	0.1CimpTOpoc	43
45		Grazing of POC by Nematodes	CpocTOnem	0.4CimpTOpoc	172
46		Grazing of POC by Ciliates	CpocTOcil	0.1CimpTOpoc	43
47	Imports	Net import of POC (import-export)	CimpTOpoc	CimpTOdoc0.3	431
48		Net import of DOC (import-export)	CimpTOdoc	CimpTOpoc0.9	388

The number of roles at every single scenario was about 3, it means that the community has 3 different trophic patterns attending the topology of the network; those can be identified as primary producers, detritivorous and consumers. Also, the link density (connectivity) remains close to theoretical maximum for a stable network (May, 1972). In this way, they are confined within a “window of vitality” (Zorach and Ulanowicz 2003; Ulanowicz 2004) where networks based on real ecosystems are framed compared to randomized networks. So, the proposed trophic web could be a mirror of the real microbial mat assayed. The Red/DC ratios (table 5) show the trend of the equilibrated systems to assimilate further perturbations.

Table 4. Results of the sensitivity analysis. Each field estimated is modified by minus (-) or plus (+) 10% and 50%.

	SI (+10%)	SI (-10%)	SI (+50%)	SI (-50%)
Total System Throughput	1.01	1.01	28.71	23.80
Effective connectivity	0.02	0.02	1.01	0.88
Effective number of nodes	0.04	0.04	2.72	2.32
Effective number of flows	0.03	0.03	1.68	1.41
Effective number of roles	0.00	0.00	0.34	0.36
Shannon´s diversity of flows	0.16	0.16	4.17	3.67
Development capacity (DC)	0.97	0.97	29.64	24.87
OVERHEAD (Φ)	0.91	0.91	27.60	24.49
Average mutual information	0.18	0.18	4.70	4.39
Hc	0.05	0.05	0.87	0.83
Ascendency	1.17	1.17	36.11	26.10
Internal Ascendency (Ai)	1.15	1.15	34.22	26.16
Redundancy (R)	1.03	1.03	30.23	23.35
R/DC	0.06	0.06	0.37	3.02
A/DC	0.22	0.22	4.07	2.44
Ai/DC	0.20	0.20	2.88	2.55
Detritivory -herbivory ratio (D/H)	0.03	0.03	7.75	5.84

The overhead (*Φ*) and total system throughput (*TST*) values also agreed with the situations proposed at every scenario (table 5), modulating the costs of maintenance at the planned scenarios. It increases when a higher predator pressure is added and is taken down when the primary production is reduced.

Table 5. Estimated parameters from Ecological network analysis of the community from SW pond at Byers Peninsula (South Shetland Islands, Antarctica). 5 different scenarios (scenario 2-6) are presented related to the estimated field data (current scenario). In the scenario 2 the primary production has been raised up a 35 %, contrastingly to the scenario 3, where a 35% of the primary production has been reduced. Also, at scenarios 4 and 5 the predation pressure has been raised up or reduced 35% of the basal model regarding to the features of ciliates, nematodes, tardigrades and rotifers. For the scenario 6 an increase of 70% of the POC in the community has been applied.

ENA parameters	Current scenario	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario6
Total System Troughput (TST)	3944	3600	4084	3458	4430	4954
Effective connectivity (C)	2,83	2,81	2,83	2,73	2,88	2,80
Number of nodes (N)	65,32	63,56	63,99	56,19	71,10	63,12
Number of flows (F)	23,12	22,60	22,60	20,59	24,65	22,54
Number of roles (R)	2,90	2,86	2,82	2,77	2,96	2,87
Shannon´s diversity of flows (H)	2,92	2,60	2,98	2,49	3,28	2,80
Development capacity (DC)	41510	37407	42619	34936	48389	52205
OVERHEAD (Φ)	31593	29462	32091	27582	35801	40265
AMI	2,51	2,21	2,58	2,13	2,84	2,41
Hc	0,41	0,39	0,40	0,36	0,44	0,39
Ascendency (A)	9918	7945	10528	7355	12588	11939
Redundancy (Red)	3558	3234	3693	3015	4076	4432
Red/DC	0,086	0,086	0,087	0,086	0,084	0,085
A/DC	0,24	0,21	0,25	0,26	0,21	0,23
D/H	3,2	3,6	2,9	2,5	3,9	0,547
FCI	0,33	0,29	0,32	0,29	0,35	3,5

The ratio of Detritivory to herbivory (*D/H*) also follows the expected at each proposed scenario (table 5) and it ranges from 3.9 (Detritivory is 3.9 times more important than herbivory) to 2.9, but the current situation *D/H* ratio is 3.6 which means almost an average state on the modified scenarios. The lost of activity (*TST*) in the scenarios 2 and 4 can be explained by a slight decrease in recycling with increasing parasitism, which is quantified by the *FCI* (table 5). The parasitism is part of a process with no-recycling pathways (table 3), which leads to a decrease in recycling activity compared to the overall activity. This effect is moderate but not negligible, as the *FCI* decreased from 32.5% to 29.2% in the scenario 2 (lower Primary production) and to 28.8% in the scenario 4 (higher predation pressure). Thus the decrease *FCI* values at scenarios 3 and 6 are almost negligible. As the *FCI* decreases with situations of over-demanded primary production in the scenarios 2, 4 whilst *TST* increases, it can be concluded that the increase in *TST* is due to higher Detritivory and parasitism activity. In the same way *AMI* values that means specialization level in the community is raising up only at scenarios where the total throughput increases also. That might denote those populations try to maximize competition effects when possible starvation periods take place. The asymmetries of *A/DC* values compared to *AMI* are explained by the maintenance of redundancy in the system (86±1% of the *DC*) (i.e. fewer of parallel pathways of equivalent importance for a carbon atom to flow from one compartment to another) that stress the importance of recycling processes within the system, faced on heterotrophic bacteria and fungi roles.

DISCUSSION

Air-mat temperature profile (figure 2) may be related with the dominance of psychrotrophic organisms at first stages of summer followed by ones more eurythermal, as it is reflected by primary producer ecological succession. This assumption is supported by PAR profile (figure 1), which means that a very effective photosynthetic apparatus is needed while snow and ice remains over the community and maintaining a narrower temperature range on the mat surface. As a result of temperature average increasing over 0 °C (figure 3), the sprays from sea accumulated during winter over the snow, are taken over by liquid water and interfere with the chemical characteristics of the community (figure 8). Evidently, those constraints limit the patchy distribution to water networked areas were nutrients supplies are swept by water. PvsI curves show the progressive adjustment of the community to forward environmental conditions. α marks the affinity of the community by the incident photons in the seasonal specific productivity of the community (Harding et al. 1982; Vandevelde et al. 1989; Frenette et al. 1993). So, the lower α value at the stage of spring ice uncovered shows that the community is trying to cope with the new environmental factors. On the other side, comparing curve shapes of spring covered and summer uncovered situations, it is obvious that the community is growing under the given environmental factors, but different P_m values (as a predictor of the integral primary production of the community). This suggests the community is at climax stage within the most favourable environmental conditions (in terms of temperature and liquid water availability) during January, reaching higher photosynthetic values. The snowpack is acting as the main feature driving the light, temperature and liquid water availability. This behaviour could be an additive effect of the phototrophs presented within the community.

The temperature effects on the community are also evident on C dynamic. Raw data of C assimilation, as CCAr fitting showed in figure 5, mark the strong influence of temperature that the process has, is good to bear in mind that those data mark the

potential capability of C assimilation without extrapolations to surface mat or isotopic signal basal levels. The coupled profile of particulate organic carbon illustrates the potential capability of the community of increasing the C budget every year. Here the temperature profile is used as start and end bulk point of the growing season, which we establish as slightly lower than 100 days per year. As attributable to the strong temperature dependence of primary producers and as the POC model shows, this community is at high equilibrium stage, season after season. Here we assume that the POC model lower confidence interval at the last days is about the same level as those for the community under frozen condition in spring time ($POC_i \approx POC_f$) and this slight difference might be assimilated by the heterotrophic bacterial fraction, those with a psychrophilic profile, of the community during winter. Here, the SO_4^{2-} concentrations may point out a bacterial activity (Villeneuve et al. 2001), but it seems to be residual. This may explain why the microbial mat appears as in stationary phase, without net growth. So, this assumption means that the recycling processes have a key role in the community, since the most of C input via primary production is consumed during summer and at fall and spring is consumed the excess margin. Fungal biomass contribution to total POC of the community estimation highlights a strong dependence to mat temperature (figure 6); this is in concordance with the psychrotrophic profiles of Antarctic fungi (Robinson 2001) exhibiting the matched occurrence of growing some days after temperature increase . The increasing standard deviations at the end of season come from the dispersal and growing dynamic of the fungal spreading, which settles where viable propagules are able to germinate. Saprophytic profiles of fungal assemblage within the community exert a total dependence of the temperature. Again, this assumption makes fungal activity an important feature together with Bacteria in C paths through the community.

Ciliate fraction is residual throughout the season but may represent the connection between the detritus fraction and upper levels in the trophic web, via bacterivory (figure 9). The describing trophic groups of ciliates in the area, taking into account rivers, lakes

and ponds, are mainly bacterivores and some other taxa of algivores and predators. Is good to keep in mind that despite the fact that cyanobacterial dominance of the biomass in benthic communities, only less than a 9% of the ciliates taxa found at Byers Peninsula are described as cyanobacterivores (Petz 2003). This highlights the niche spreading within the community. In this way, *rotifers* seem to be another important C path to upper levels via bacterivory (Arndt 1993). *Tardigrade* fraction remains stable throughout the season and thus they may play a key role in the succession between Chlorophyte and Cyanobacteria, those switch from a mostly phytophage (E. Rico pers. comm.) to a detritivorous profile. Tardigrades feeding on Chlorophyte favour the prevalence of Cyanobacteria due to tardigrades are getting out Cyanobacteria competitors. Together with warmer conditions in December, the harmonizing effects of snow depletion and tardigrade feeding habits mark the Chlorophyte-Cyanobacteria succession as main phototrophs. Those matching events are reflected in figure 10 in the decrease of primary production between the two first sampling measures. Again, figure 10 points out the possible disengagement of prey-predator dynamic between cyanobacteria, tardigrades and nematodes.

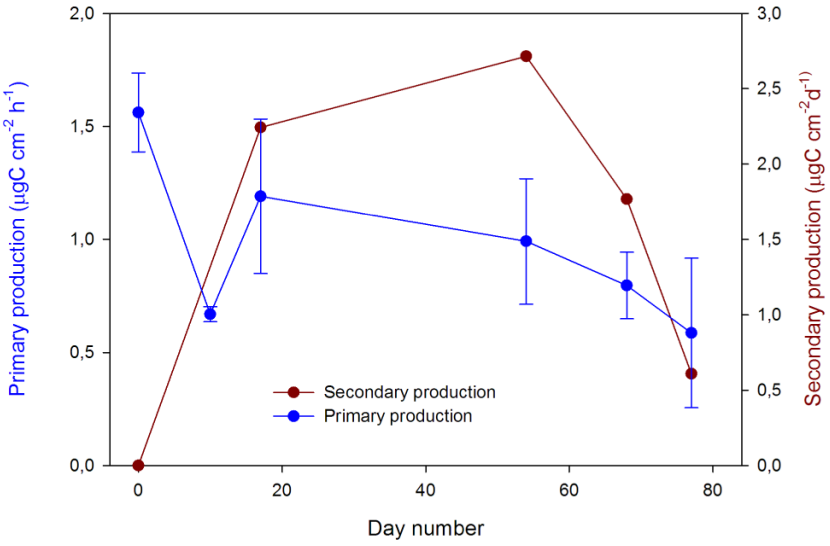

Figure 10. Dynamics of Primary and secondary production estimations at SW Pond (Byers Peninsula, South Shetland Islands, Antarctica).

Usually, the most negative values of δ ^{13}C signature in organisms are generally correlated with diffusive CO_2 entry, while more positive values relate to either presence of carbon concentration mechanisms (CCMs) in primary producers, or diffusive limitation of

organic to the supply organism, or a combination of the two (Raven et al. 2002b; Raven et al. 2002a; Kevekordes et al. 2006). Here, the average $\delta^{13}C$ signal (-12.08±0.34) denotes a non oligotrophic environment and a C turnover provenance maybe by CO_2 diffusion from the deepest layers of the mat, where respiration processes are assumed. But DOC $\delta^{13}C$ signal is slightly more positive denoting this fraction as the most important turnover feature. There is statistically difference between $\delta^{13}C$ signal of mosses (-23.16±0.46) and the rest of phototrophs assayed within the mat (cyanobacteria and diatoms) (ANOVA $P<0.001$ Bonferroni t-test). So, although they are tangled up within the cyanobacterial matrix, most probably they are not drawn in the community as possible food; but as a possible dissolved organic carbon resource via exudation. On the other hand, $\delta^{15}N$ diatoms signal leaves them out of the trophic web by predation and its biomass is probably reintroduced by a microbial loop as DOC and DON, this is consequence of a lack of predators with a chewing pharynx big enough to break diatom valves; a general description of the diatoms genus observed in the community (*Navicula* spp., *Nitzschia* spp., *Achnanthes* spp. and *Pinnularia* spp.) are ranged 25-70 μm and the biggest predators are in 400 μm with some, but scarce, bigger exceptions of nematodes about 800-1000 μm. Assuming those restrictions, figure 11 displays the trophic network proposed for the community studied.

In their review, Raven and co-workers (2008) pointed out that for algae and cyanobacteria, at a constant inorganic carbon supply, the affinity for inorganic carbon increases when nitrogen (as nitrate) is limiting growth (Raven 1991a, b; Raven et al. 2005). However, when nitrogen is supplied as ammonium there is a decreased affinity for inorganic carbon when nitrogen is limiting (Raven et al. 2005). The present study reveal a non N-limiting ecosystem (figure 12 and figure 8), DIN is driven by ammonium-nitrate presence in a ca. 75:25 ratio at the beginning of the season. This ratio switches when ice and snow cover thaws. At that point, nitrate is the largest contribution to DIN and the affinity for inorganic C is even bigger. Besides, the assumption of ecological succession

from green algae, while the whole community remains almost frozen, to cyanobacteria as most important photosynthetic organisms is corroborated by microscopic observations.

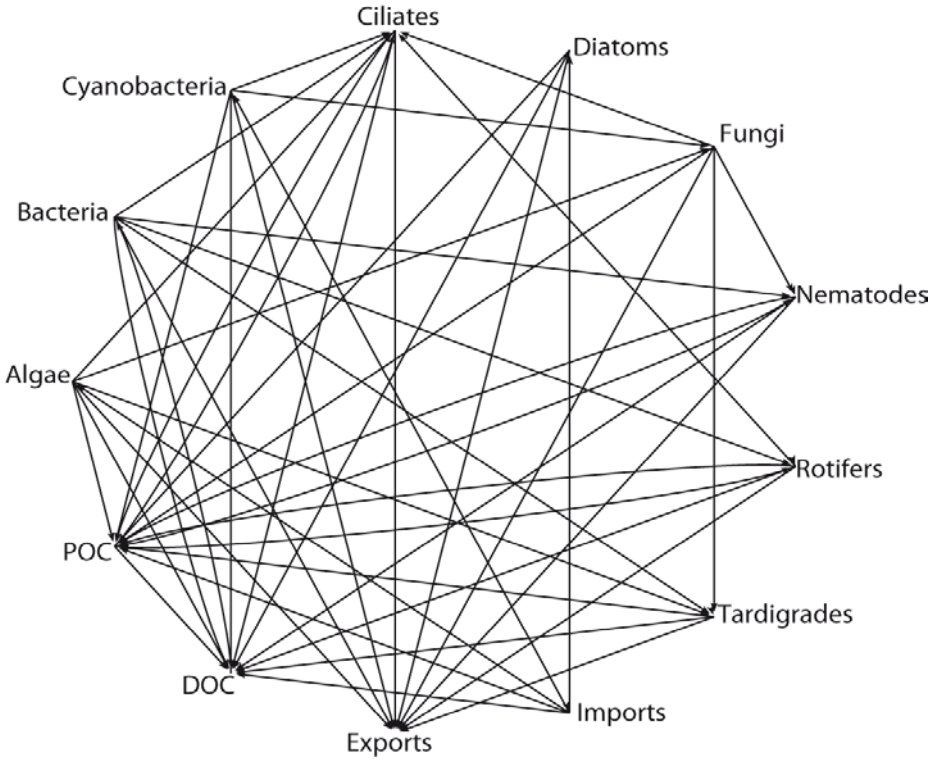

Figure 11. Proposed trophic web at SW pond microbial mat (Byers Peninsula, South Shetland Islands, Antarctica).

The network analysis presented here shows a stable trophic web that mirrors a non randomized model (Zorach and Ulanowicz 2003). Hence the information flows between the nodes of the web, presented as C flows, has an overhead large enough to resist impacts as introduction of new species. Thereafter, the permanence of these communities, based on a cyanobacterial matrix, provides a proper environment where non colonizing species are able to maximize its fitness and counteract the harsh physical conditions where they are settled. The influence of physical and biological C loss processes as respiration or lixiviation seems not to impact greatly on the net C (figure 5). The temperature effects on the community mark the beginning of the growing season as water availability via snow and ice melt. This is the point when a chain of matched events takes place. The less water availability together with the lack of a cyanobacterial matrix could

explain the areas coated by microbial mats are not wider in extent and confined to flowing water surroundings. So, in bare-field soils the communities found are absolutely different and presumably with different trophic networks (Convey and Stevens 2007). The proposed model prompts those ecosystems as the main C promoters on Antarctic freshwater ecosystems that provide dissolved elements to associated ecosystems like rivers and lakes (Tang et al. 1997). The FCI values are surprisingly high compared to a modelled atoll by Niquil and co-workers (Niquil et al. 2006), but similar in a model developed for Lake Biwa (Niquil et al. 2011), where the role of fungi was well determined, so that prompts our model also displays a high dependence on detritivory and turnover cycles, that enhance the circulation of carbon between the compartments of upper trophic levels. The absence of quantification, and as a consequence of definition of an interval of confidence, prevents us from performing any statistical test on the differences observed in the Ecological Network Analysis index values. However, the comparison with other aquatic systems gives an indication of the relative effect of the simulations.

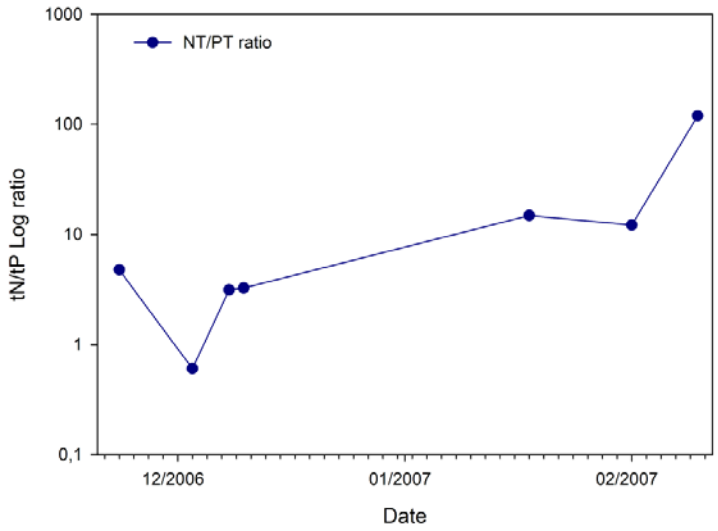

Figure 12. tN/tP ratio of SW Pond (Byers Peninsula, South Shetland Islands, Antarctica) during 2006-07 Antarctic summer season.

The great amount of POC accumulation of this kind of communities may fail the occurrence of prey-predator meetings. That could be translated as a decrease on specialization and asymmetry of the system between upper trophic levels and the lowest. That situation has been previously described as a lack of stability by Rooney at al. (2006). So, as far as the inferred results from the different scenarios permit to take conclusions,

we are able to assume that the microbial community, SW Pond at Byers Peninsula, is very stable and probably at the top of its development, where recycling processes are crucial in the maintenance of its stability (Figure 11).

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Conclusiones Generales/General conclusions

1. Cada tapete microbiano, tiene una ontogenia propia e individual. Así, la composición de cada tapete no es consecuencia de una sola o varias variables fisicoquímicas, tampoco de la combinación de varias variables ambientales, si no que es necesario incluir en los análisis tanto variables ambientales como del medio fisicoquímico en el que se desarrollan. Esta combinación de variables de distinta escala temporal y espacial junto con eventos ecológicos sucedidos en cada comunidad, como pueden ser invasiones, periodos de sequía, de frío extremo, etc.… pueden explicar mejor la composición y estructura de cada comunidad.

2. La composición de productores primarios de las comunidades bénticas de la Antártida marítima son muy sensibles a los cambios de temperatura. Así, las temperaturas que experimentan dan lugar a cambios en la composición de las comunidades y a distintos ratios de sus actividades fisiológicas, entre las que destacan la fotosíntesis y la asimilación de distintos compuestos del N.

3. Las tasas de fijación de nitrógeno y de asimilación de carbono junto con la sensibilidad a los cambios de temperatura sugieren que las comunidades bénticas de cianobacterias en zonas polares no se encuentran en su óptimo fisiológico de temperatura. Así, estas comunidades aprovechan las condiciones ambientales del verano para conseguir acumular el máximo de biomasa posible, cuando las condiciones se acercan más a sus óptimos de temperatura. Por estos motivos podríamos denominar a estas comunidades como psicrótrofas. Sin embargo, las comunidades criosestónicas exhiben crecimientos explosivos en cortos periodos de tiempo cuando el hielo aún permanece, exhibiendo un comportamiento psicrófilo.

4. En las comunidades bénticas polares la mayor parte de biomasa está concentrada en forma de cianobacterias, sin embargo, este C no pasa directamente a los niveles tróficos superiores vía depredación, si no que son los procesos saprofíticos la principal vía de reciclado de materia.

5. Los análisis de las redes tróficas sugieren que la comunidad denominada *SW pond* se encuentra en su máximo de desarrollo y que alcanza su clímax cada verano alrededor del mes de Enero. Así uno de los eventos físicos más importantes es la desaparición de la cobertura de nieve-hielo acumulada sobre ella durante el invierno. Este proceso físico desencadena una serie de eventos que dan lugar a una sucesión ecológica dentro de la comunidad regulada principalmente por procesos ecológicos.

6. La estabilidad de los valores de carbono orgánico particulado en los tapetes de cianobacterias apunta a que la comunidad se encuentra en una fase estacionaria, sin crecimiento neto. Esto significa que los procesos de reciclado tiene un papel fundamental en el desarrollo de la comunidad. Además, estos procesos de reciclado exhiben una dependencia total de la temperatura y por lo tanto sólo pueden darse durante la época estival. Estos procesos de reciclado son esenciales en el mantenimiento de la estabilidad del sistema.

7. La matriz de cianobacterias proporciona una estructura física en la que se desarrollan el resto de organismos que componen las comunidades con capacidades fisiológicas mas restringidas en cuanto a sus óptimos de temperatura. Estas otras especies maximizan así su *fitness* y encuentran un medio adecuado para desarrollarse en esas condiciones ambientales.

8. Las relaciones ecológicas dentro de cada comunidad adquieren mayor relevancia en comunidades en las que los organismos que las componen están cercanos a sus límites fisiológicos. Así, pequeños cambios en su historia biológica y en las

condiciones en las que se encuentran pueden dar lugar a composiciones totalmente distintas en estadios climácicos o próximos al clímax ecológico.

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